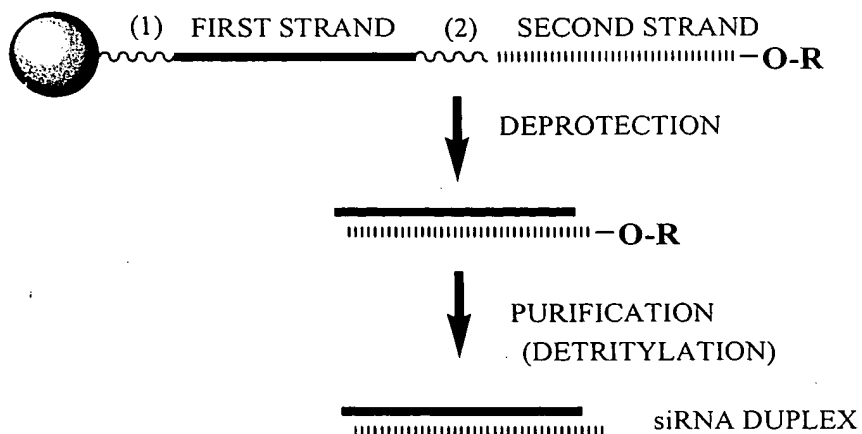


**Figure 1**

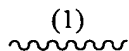


= SOLID SUPPORT

R = TERMINAL PROTECTING GROUP

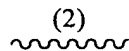
FOR EXAMPLE:

DIMETHOXYTRITYL (DMT)



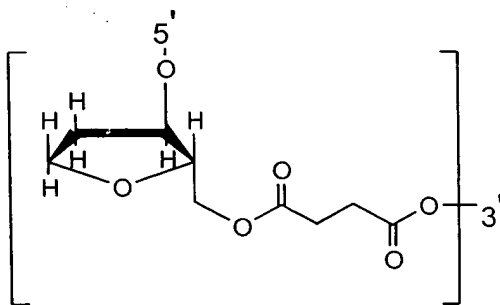
= CLEAVABLE LINKER

(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR  
 INVERTED DEOXYABASIC SUCCINATE)

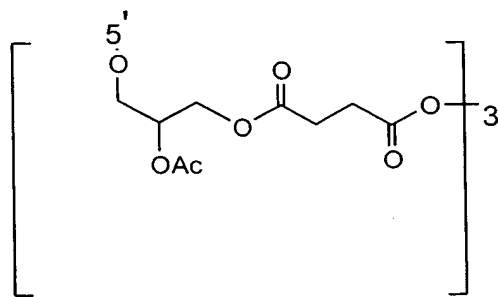


= CLEAVABLE LINKER

(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR  
 INVERTED DEOXYABASIC SUCCINATE)

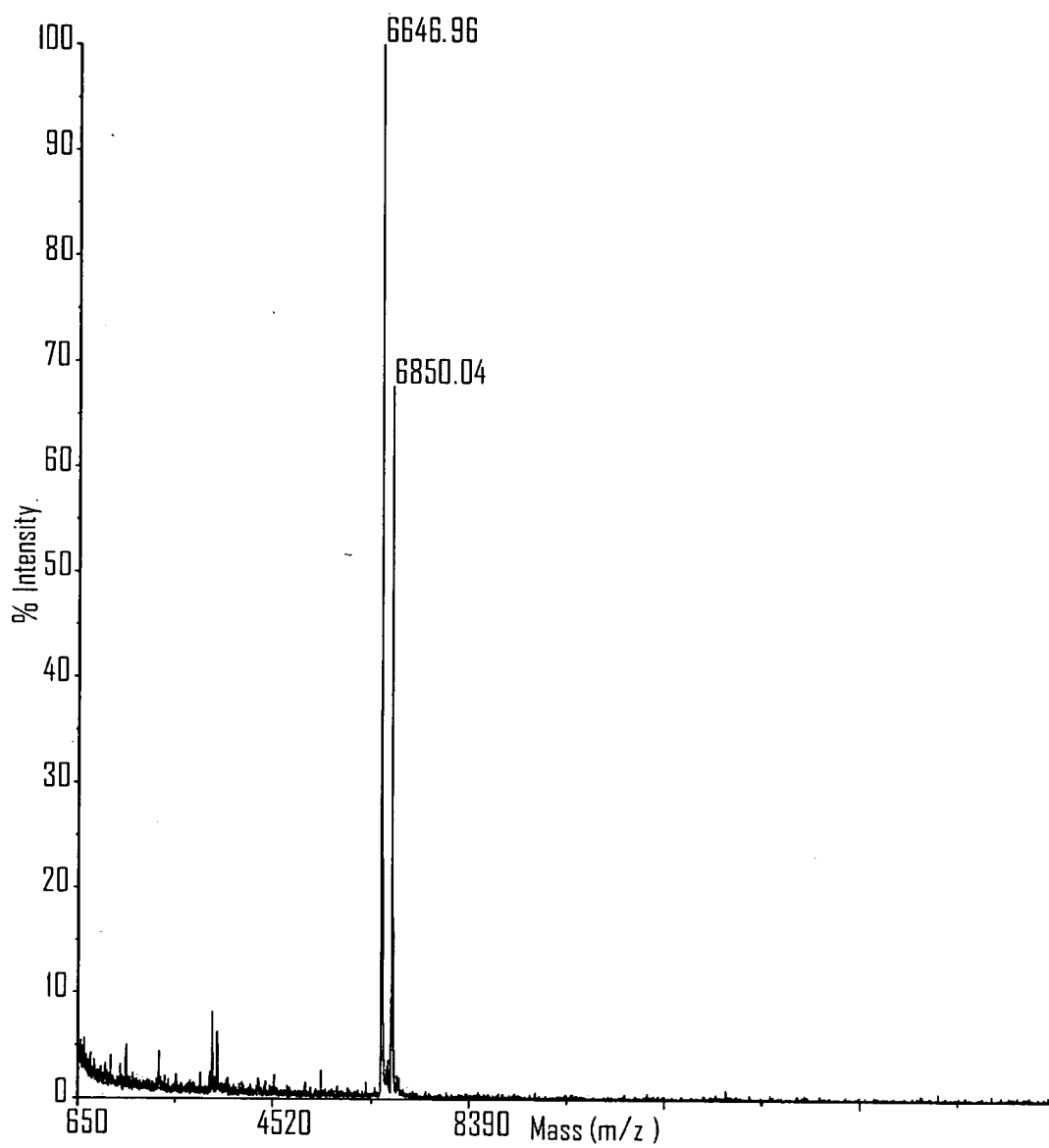


INVERTED DEOXYABASIC SUCCINATE  
 LINKAGE



GLYCERYL SUCCINATE LINKAGE

*Figure 2*



## Figure 3

5'-CGUACGCGGAUACUUCGATT (SEQ ID NO: 394) 3'-TTGCAUGCGCCUUAUGAAGCU (SEQ ID NO: 395)	$T_{1/2} = 15 \text{ seconds (control)}$
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-TXGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 397 )	$T_{1/2} = 138 \text{ min}$
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-TDGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 398 )	$T_{1/2} = 3.7 \text{ days}$
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-XTGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 399 )	$T_{1/2} = 72 \text{ minutes}$
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-LTGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 400)	$T_{1/2} = 40 \text{ days}$
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-tTGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 401)	$T_{1/2} = 32 \text{ days}$

G, A, U, C = Guanosine, Adenosine, Uridine, Cytidine

T = Thymidine

Lower Case = 2'-deoxy-2'-fluoro

S = phosphorothioate

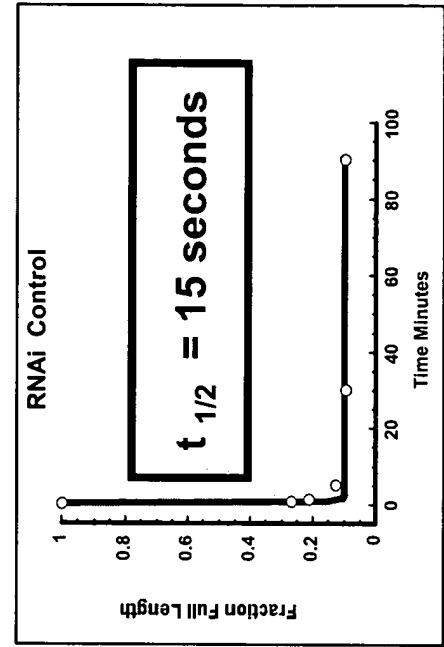
B = inverted deoxyabasic

D = inverted Thymidine

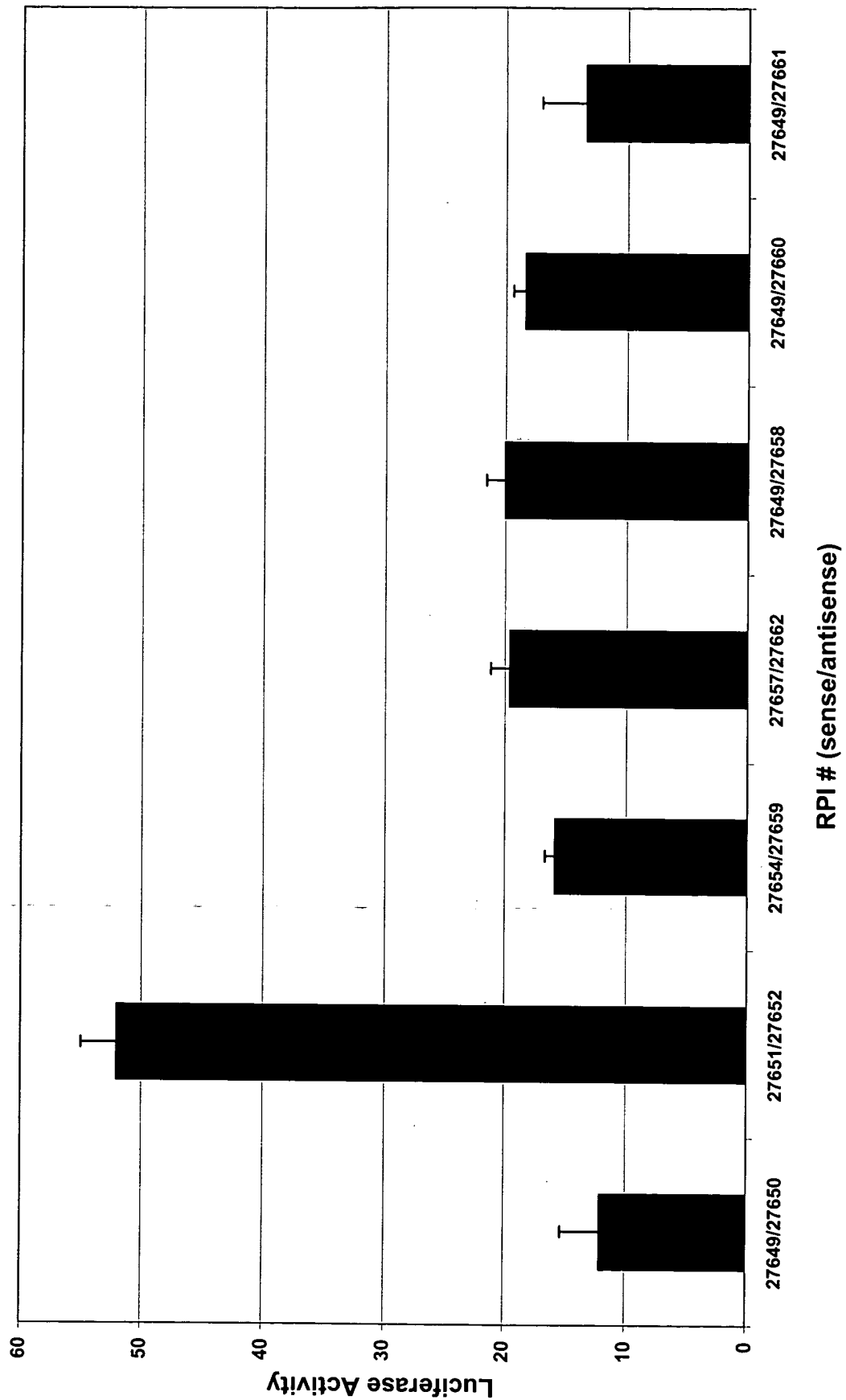
X = 3'-deoxy Thymidine

t = L-thymidine

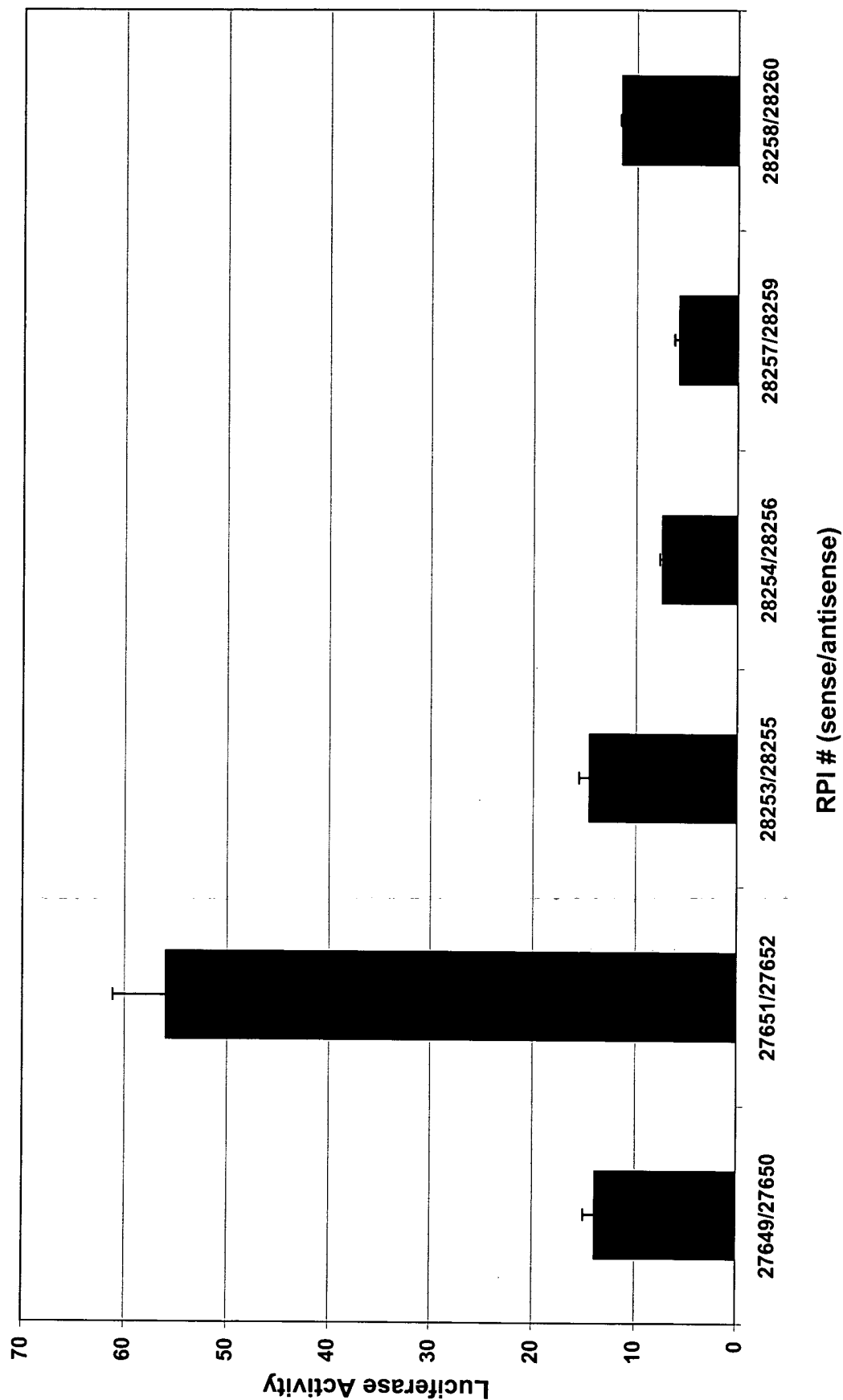
L = Glyceryl moiety



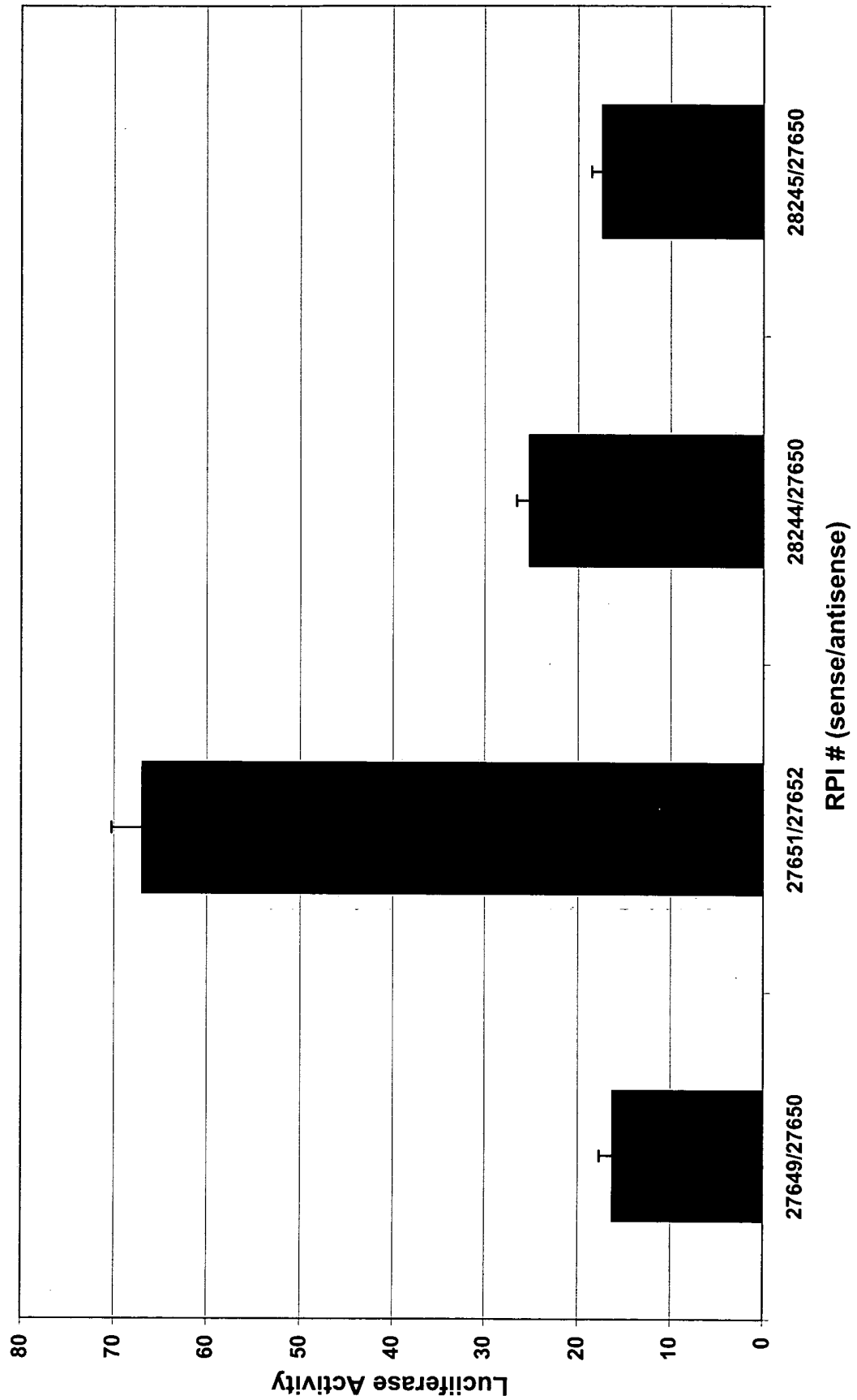
*Figure 4*



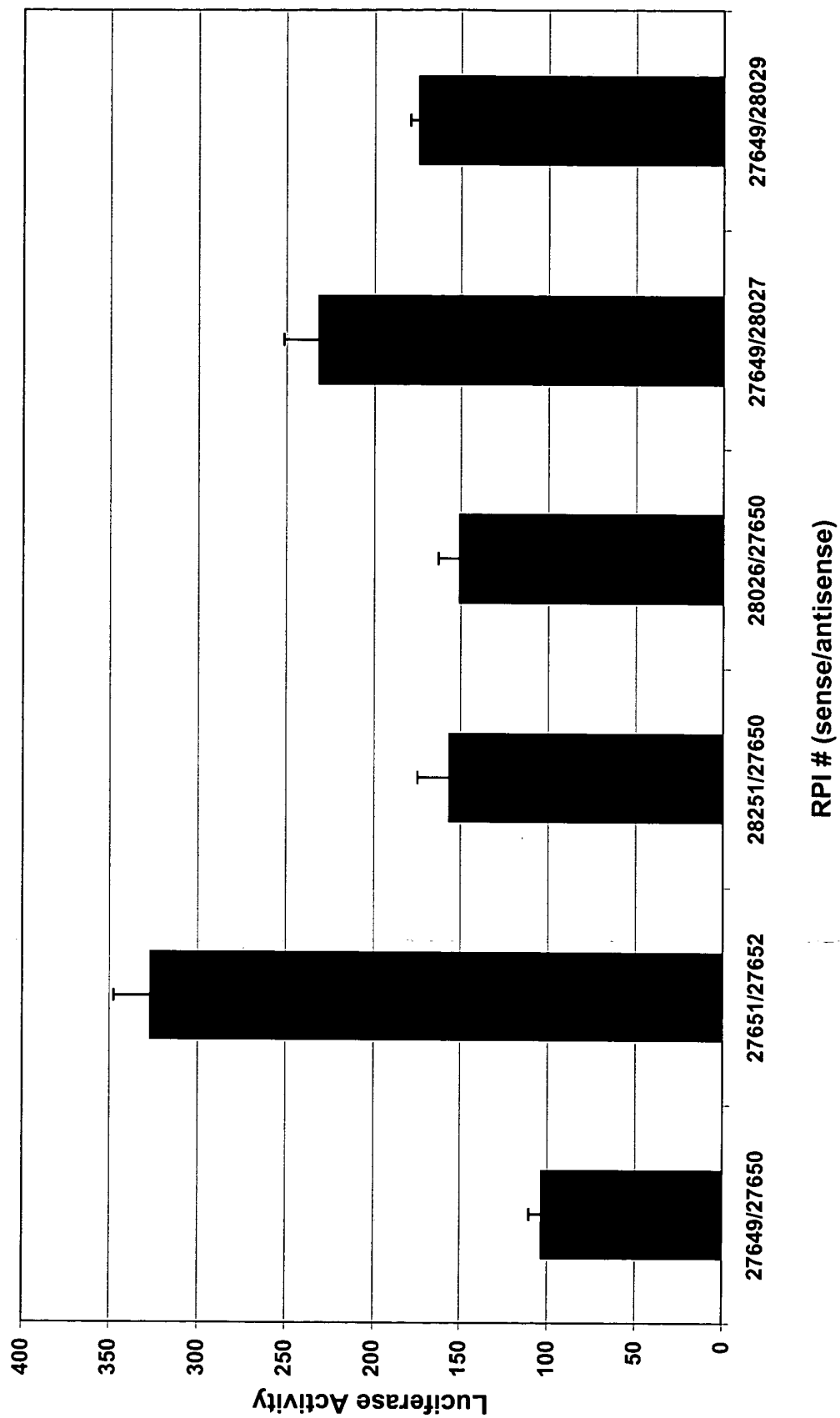
**Figure 5**



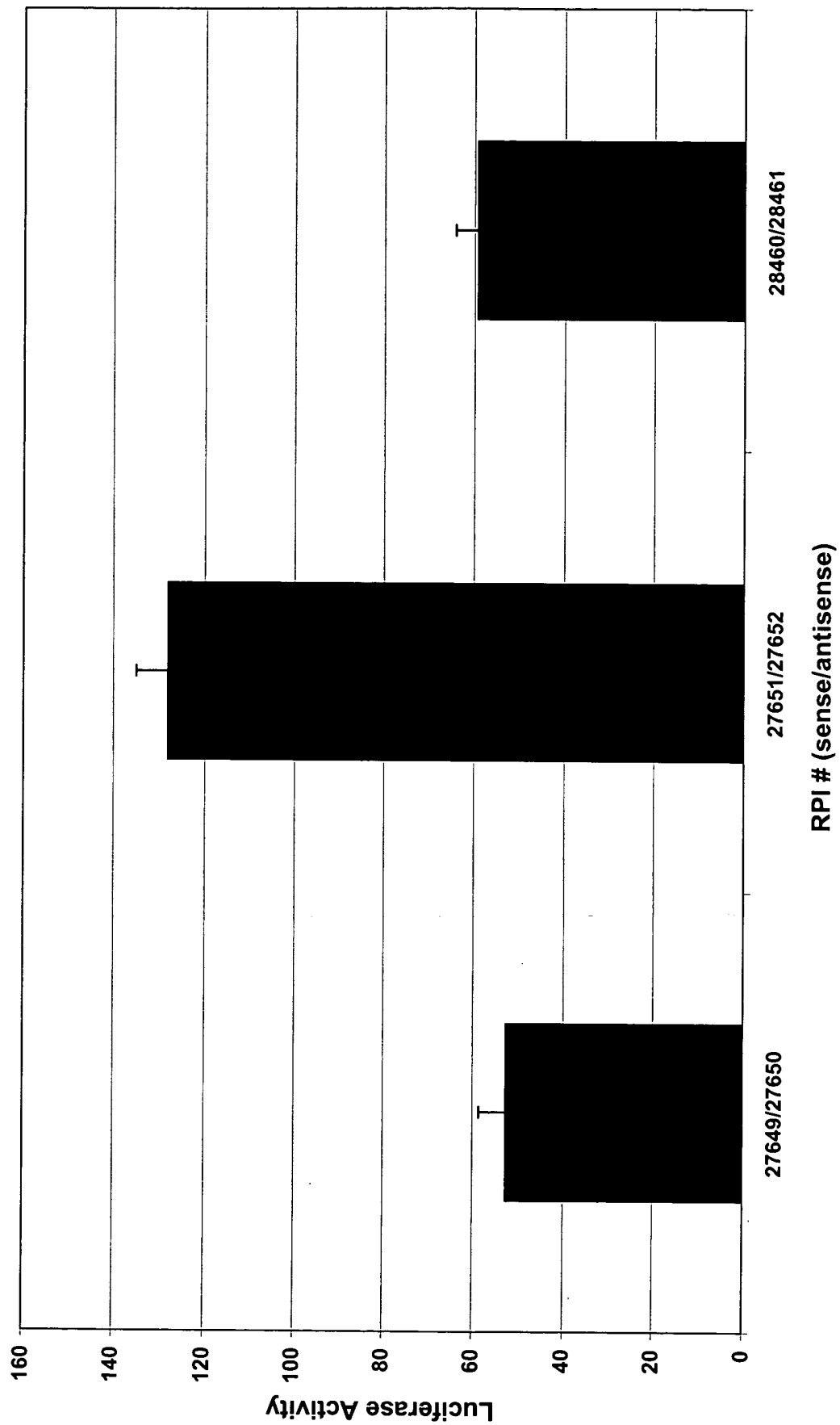
**Figure 6**



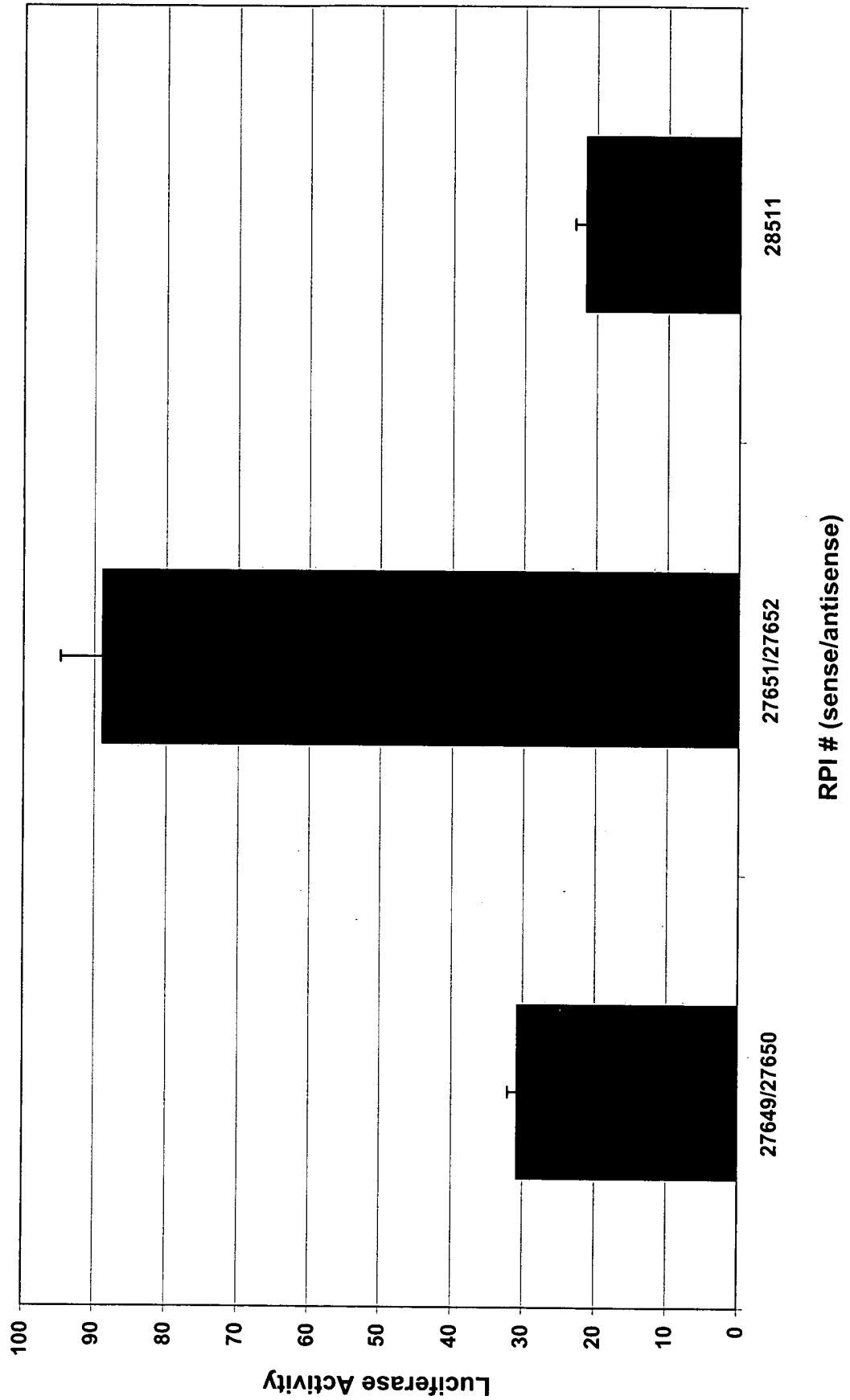
**Figure 7**



**Figure 8**



**Figure 9**



**Figure 10**

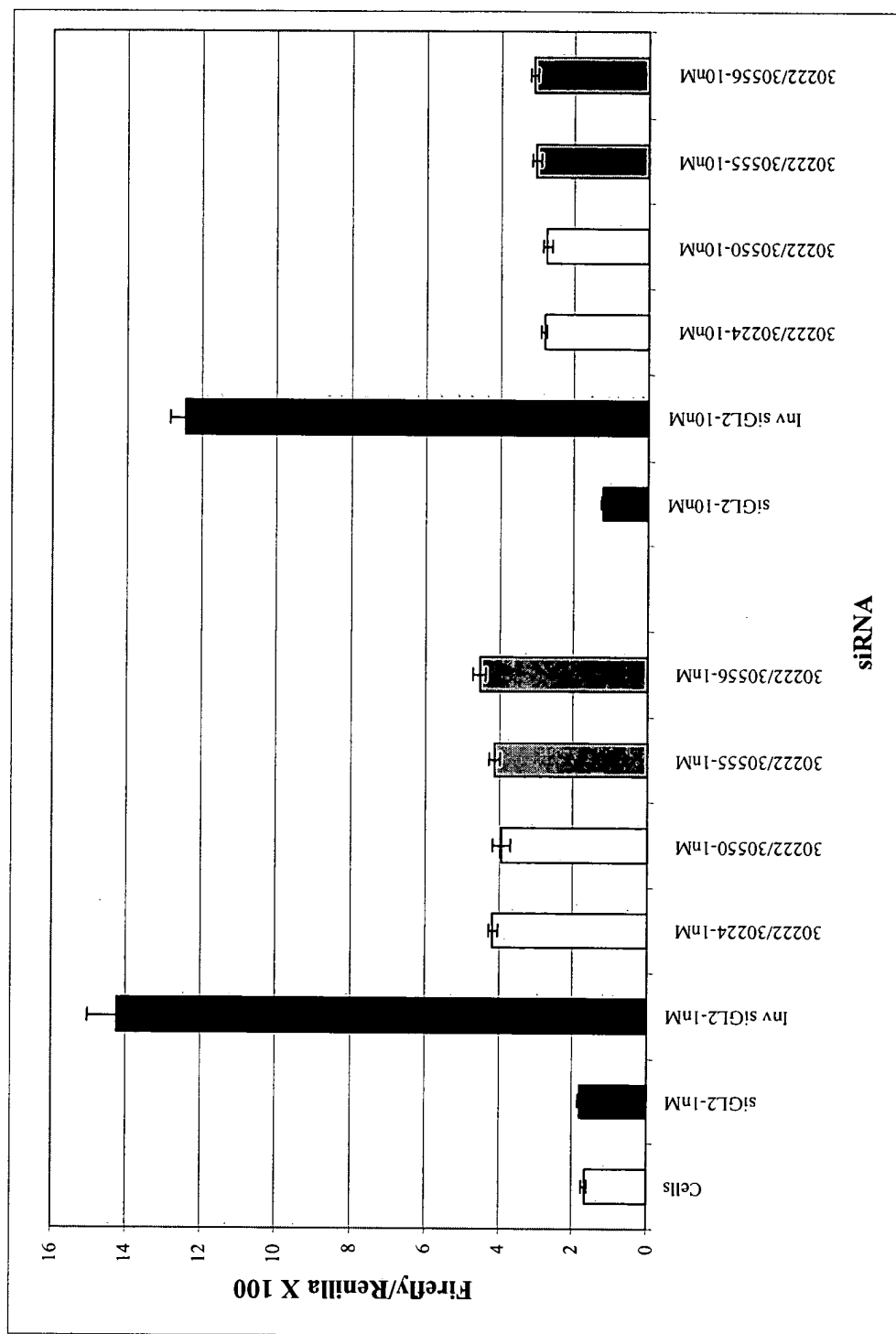
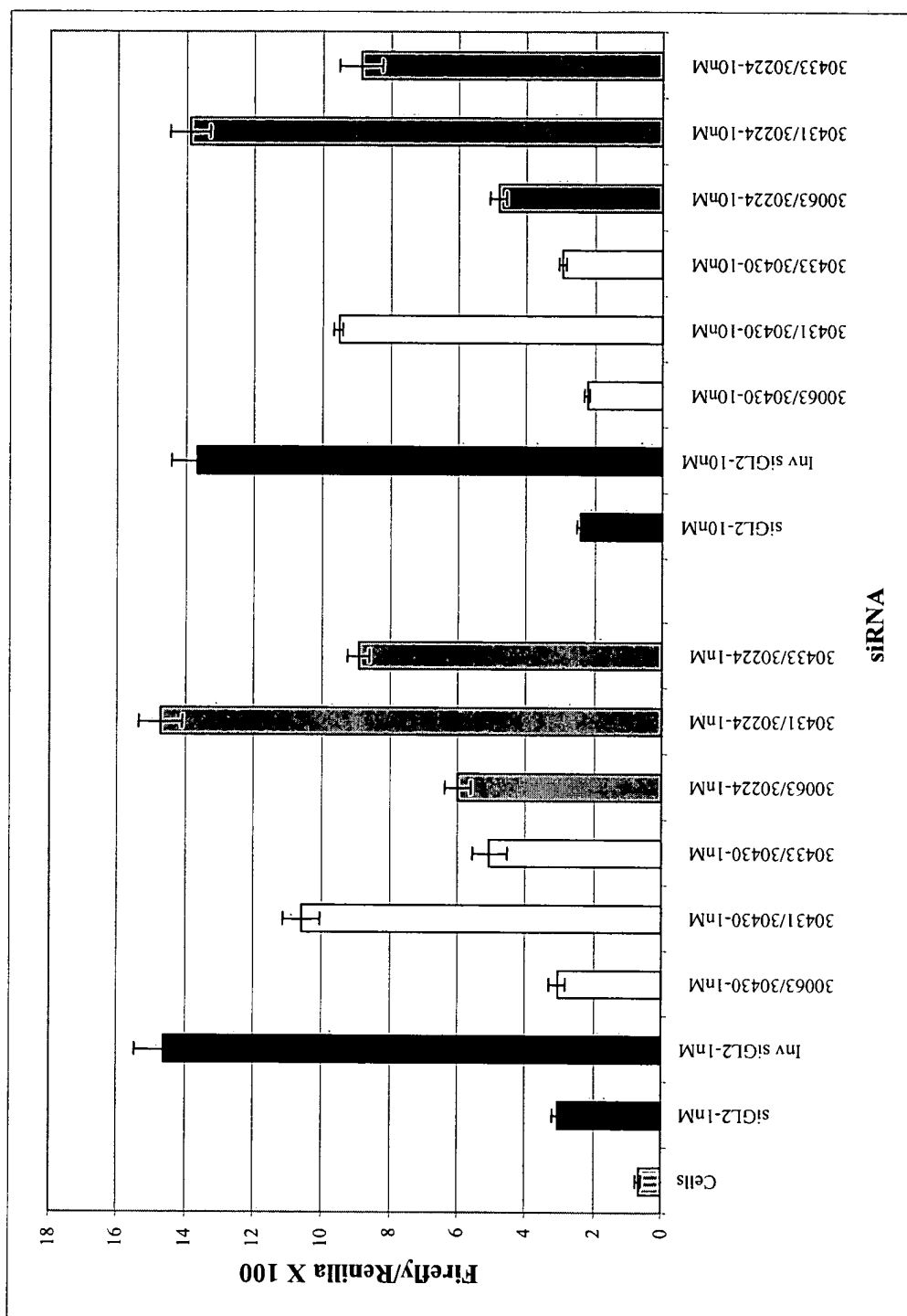
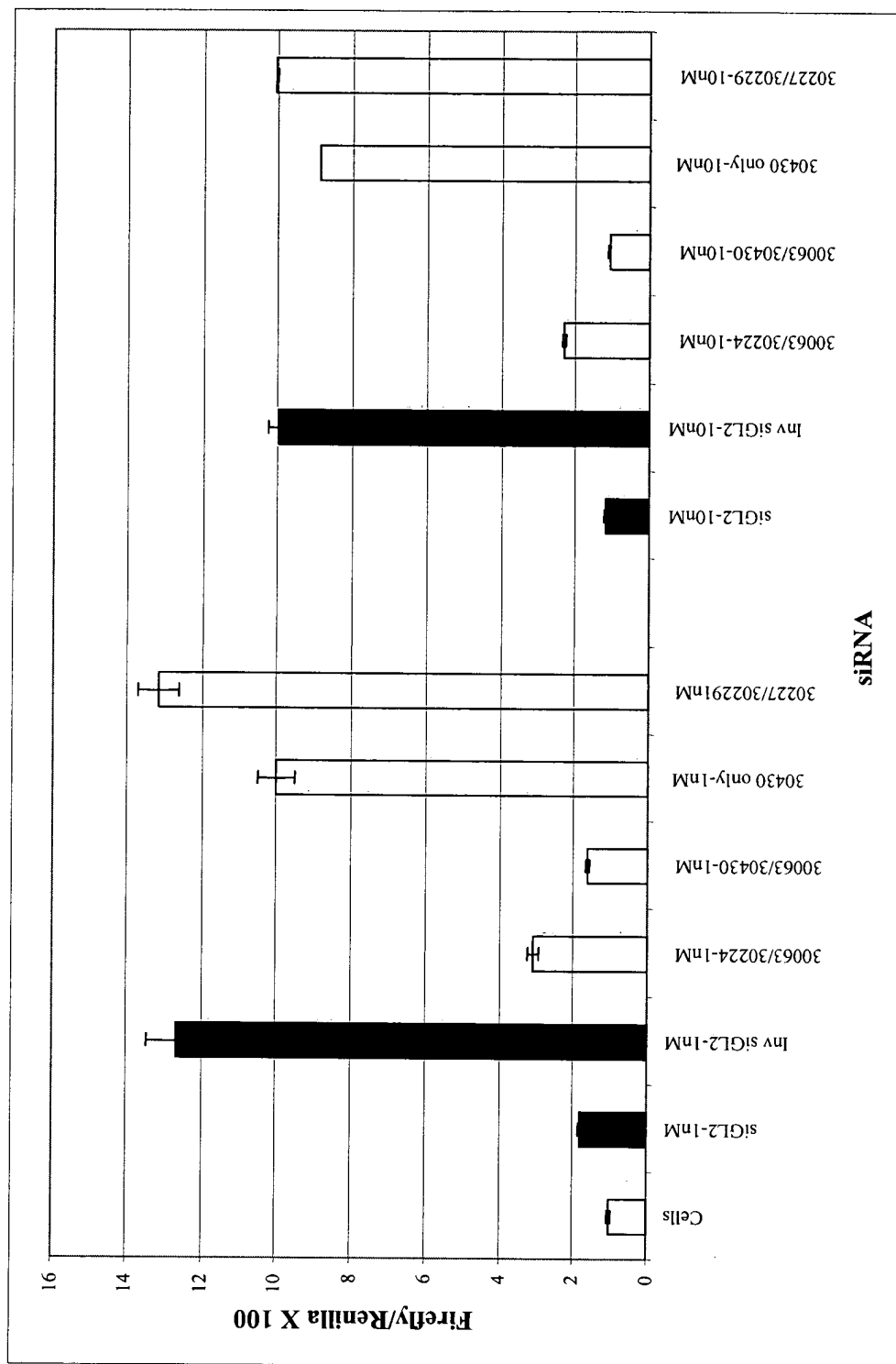


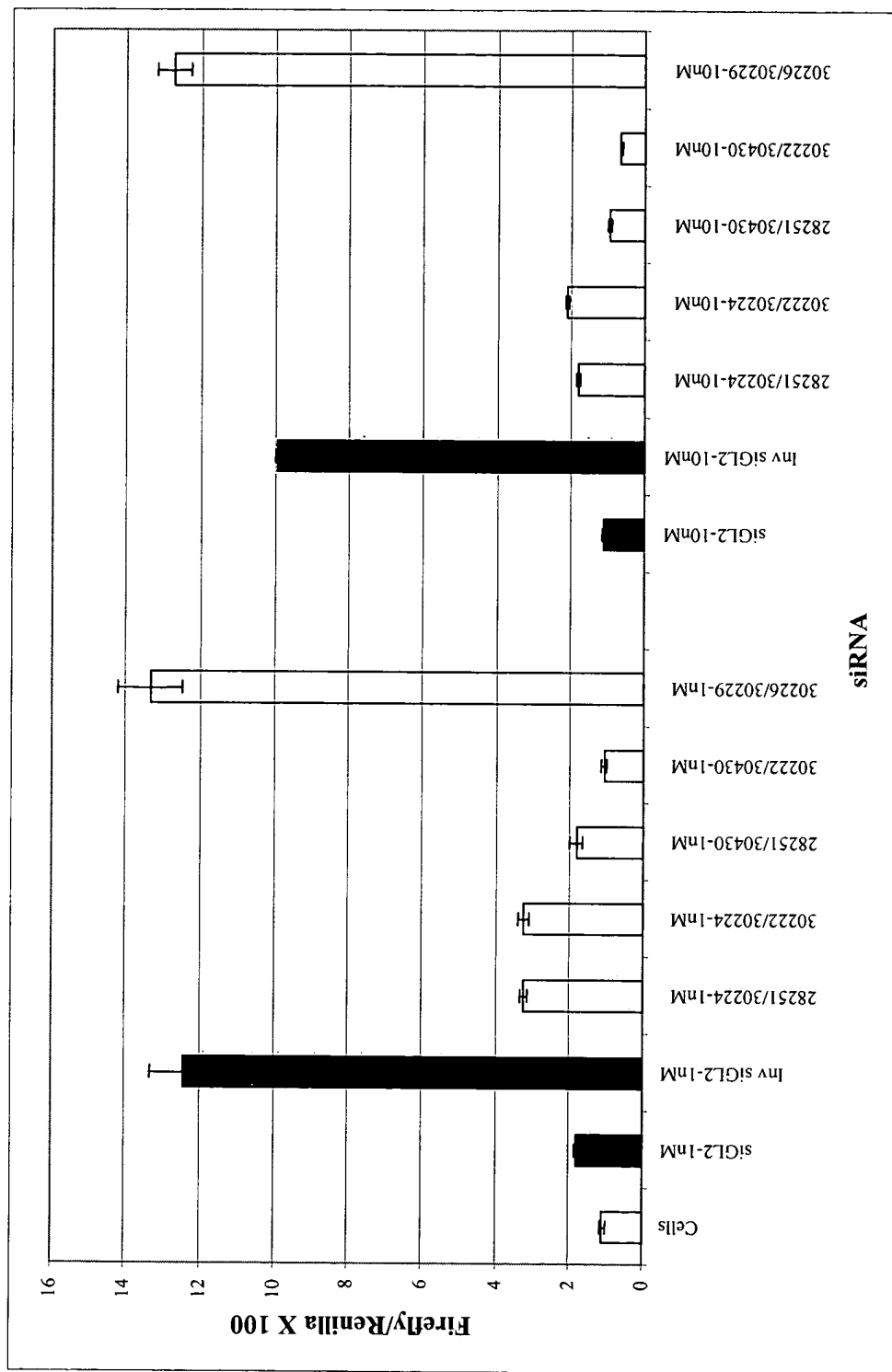
Figure 11



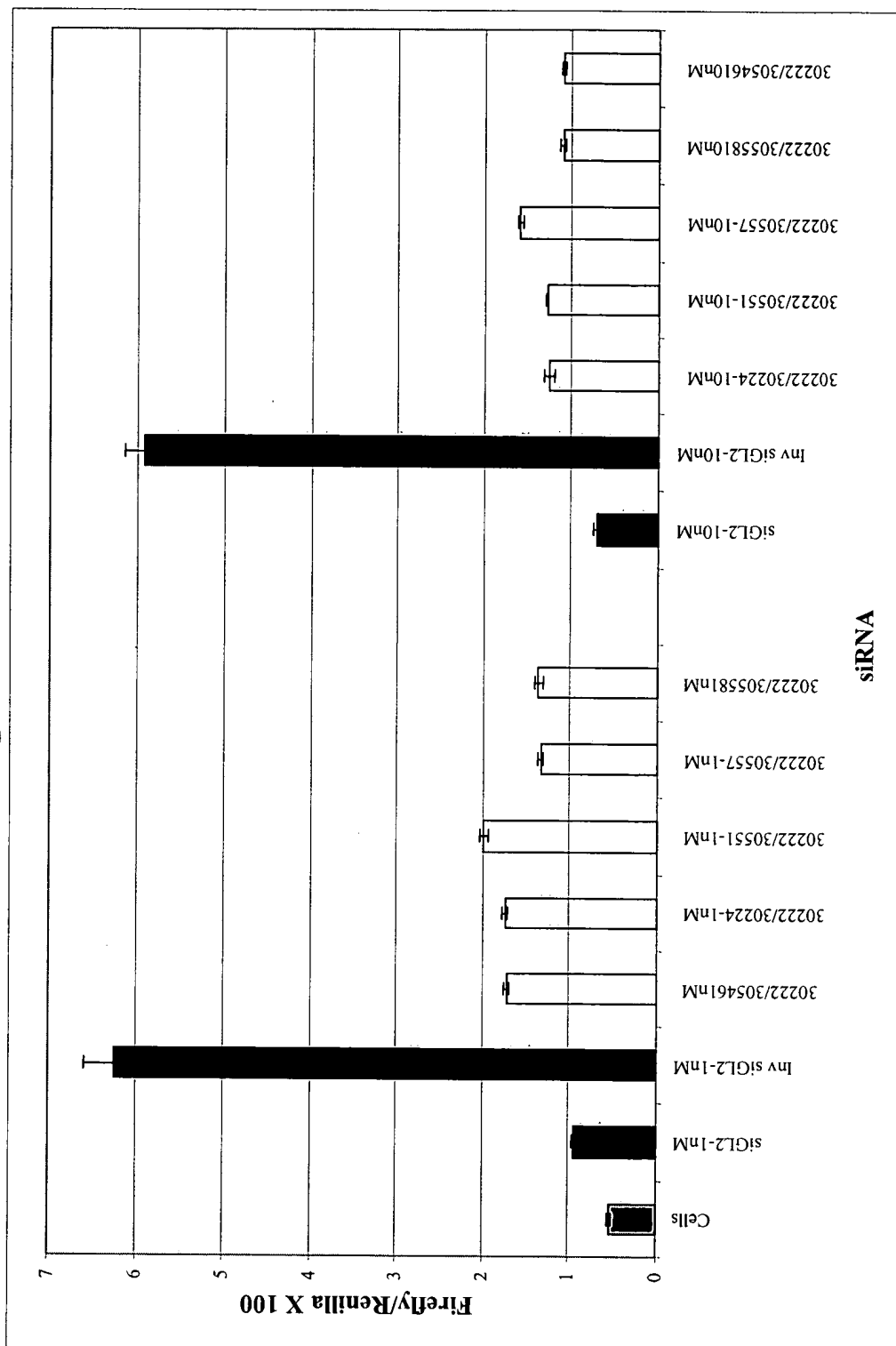
**Figure 12**



**Figure 13**



**Figure 14**



**Figure 15**

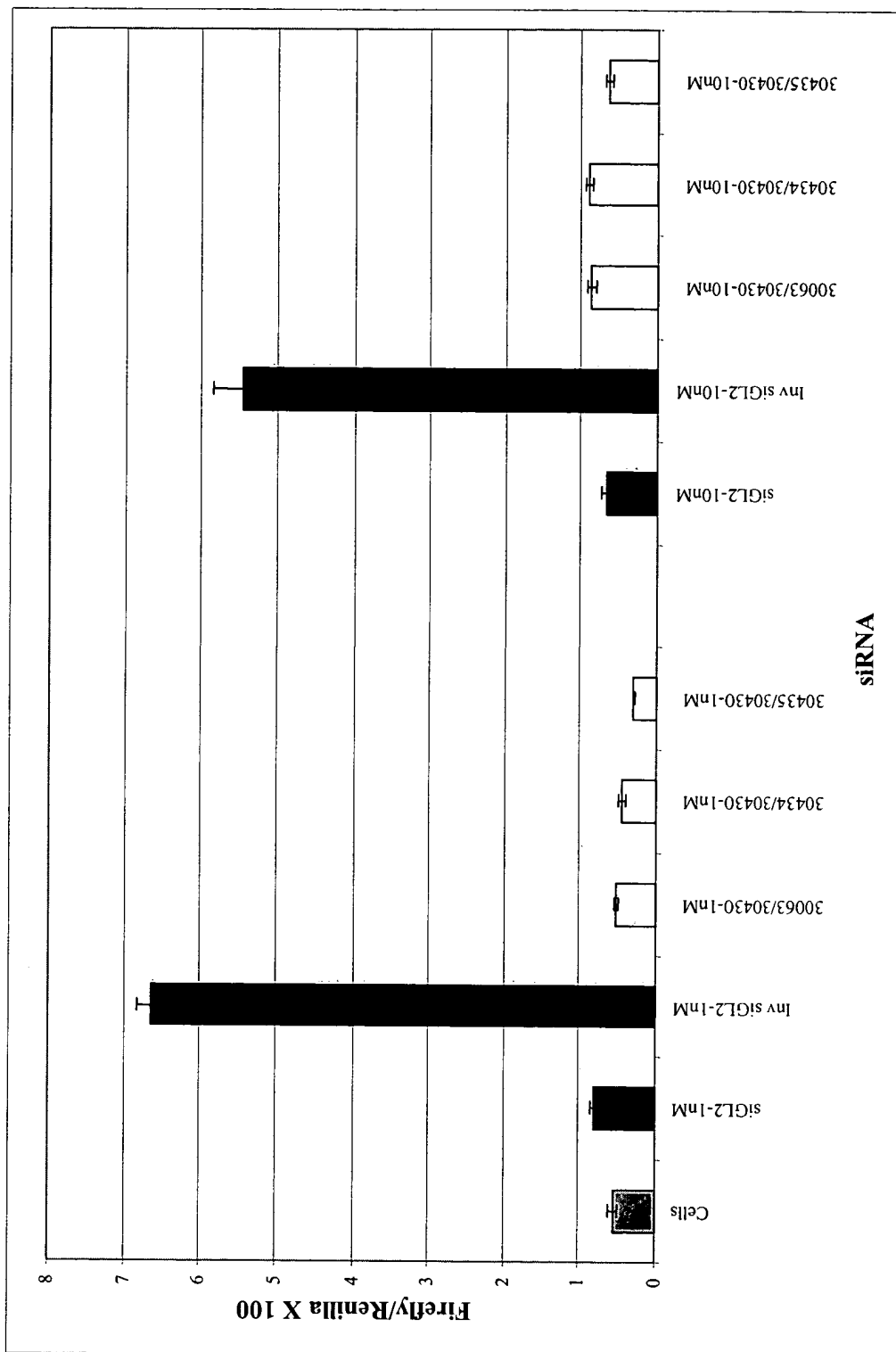
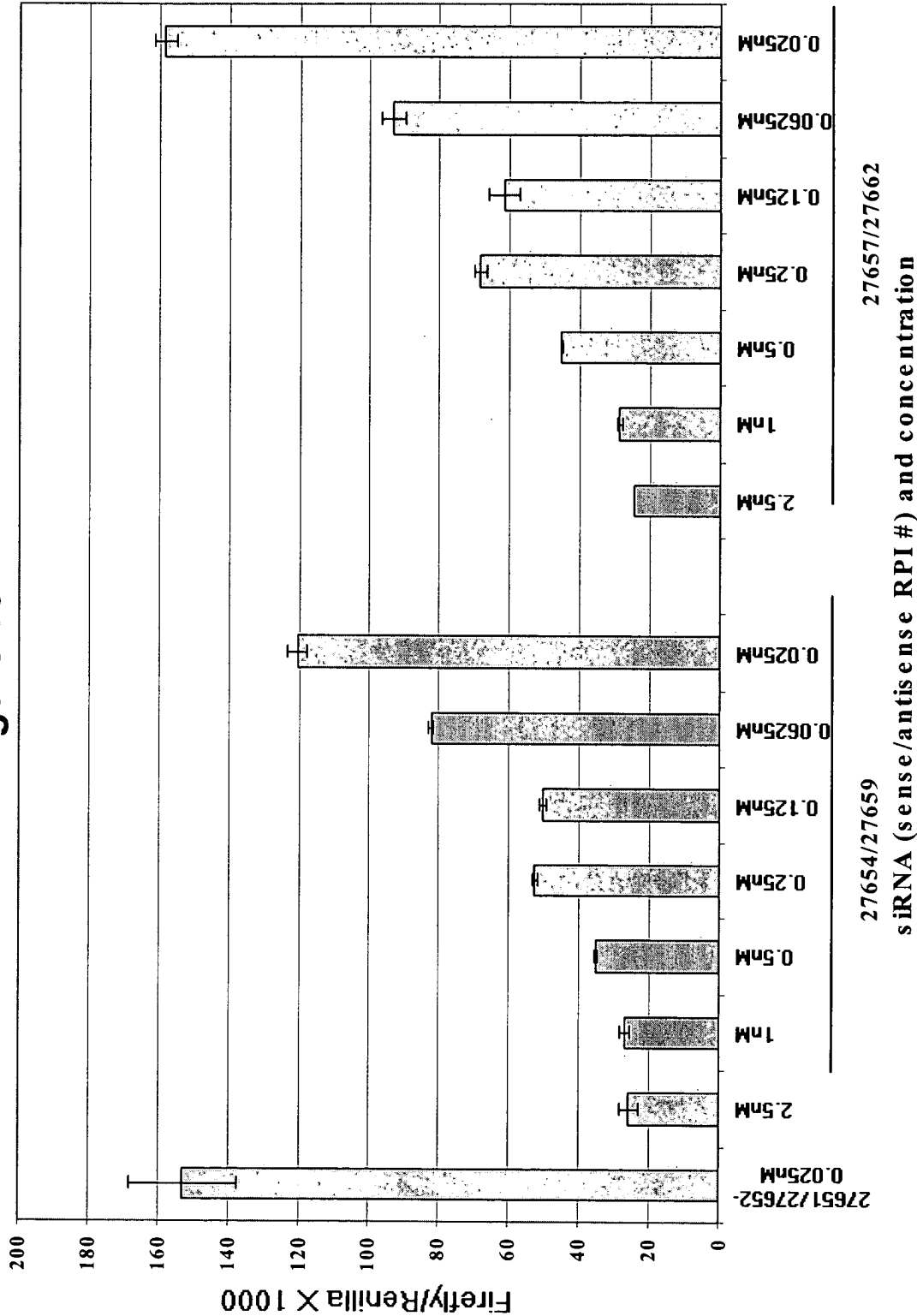
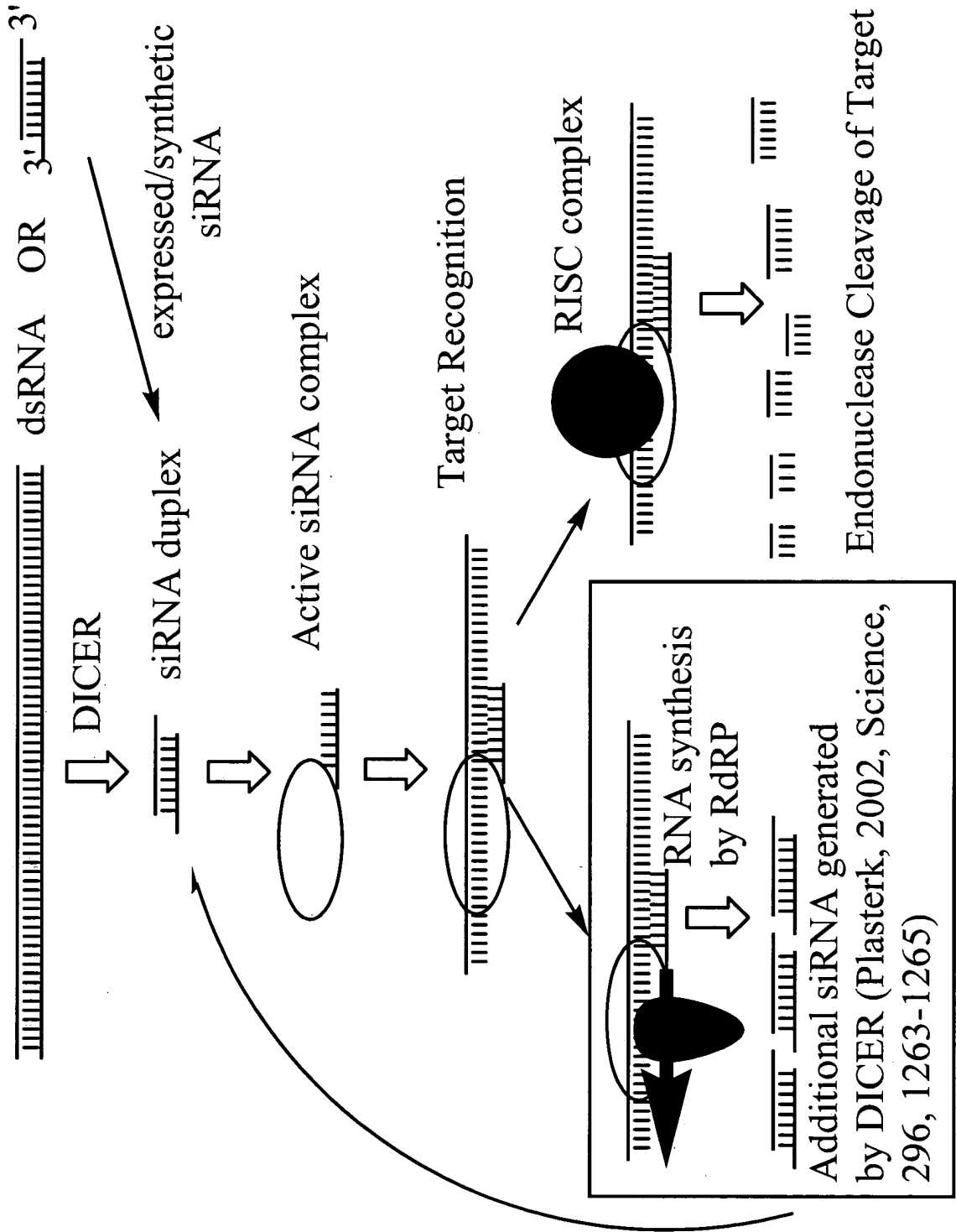


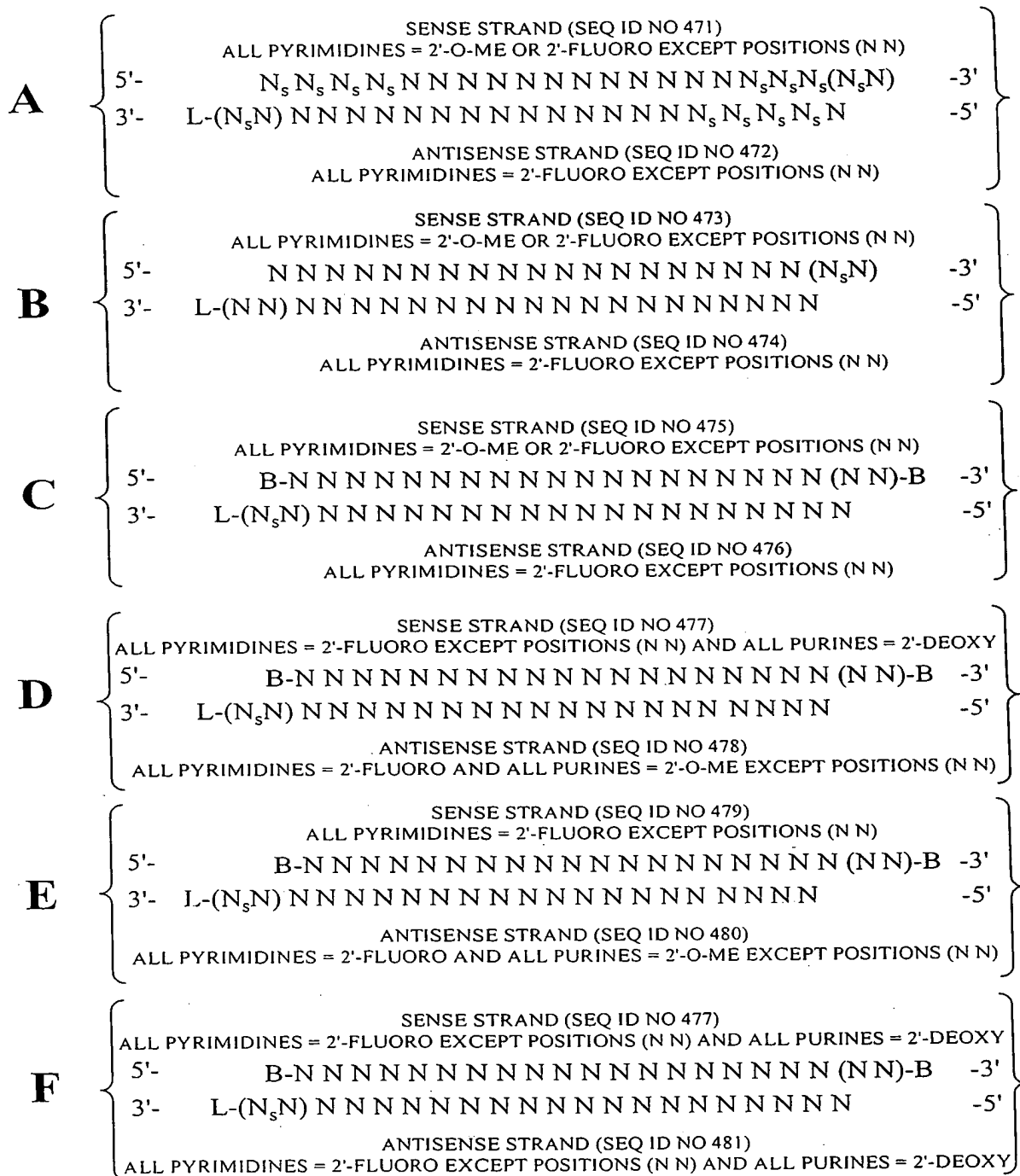
Figure 16



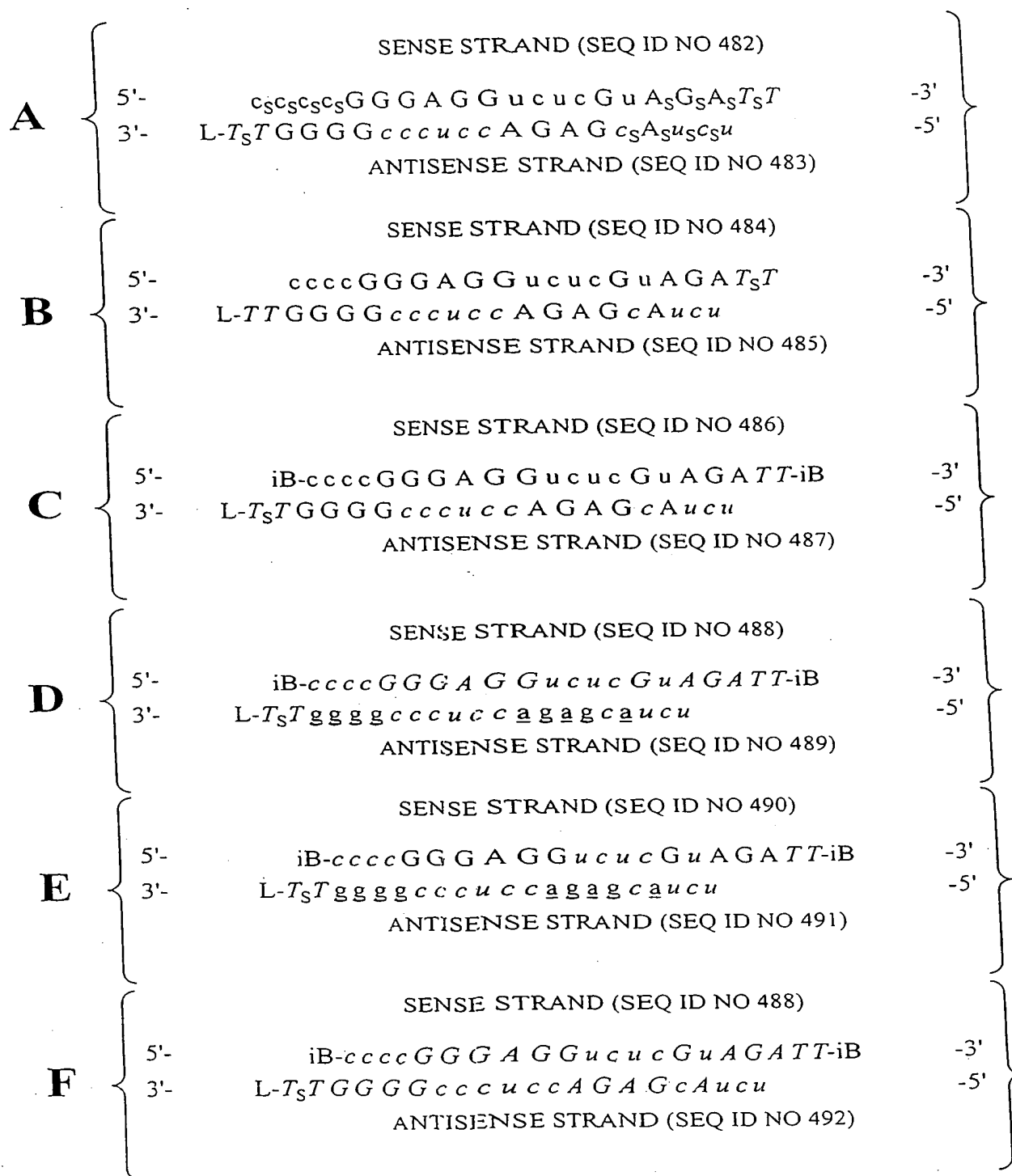
**Figure 17**



**Figure 18**

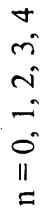


POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES  
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT  
L = GLYCERYL MOIETY THAT IS OPTIONALLY PRESENT  
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

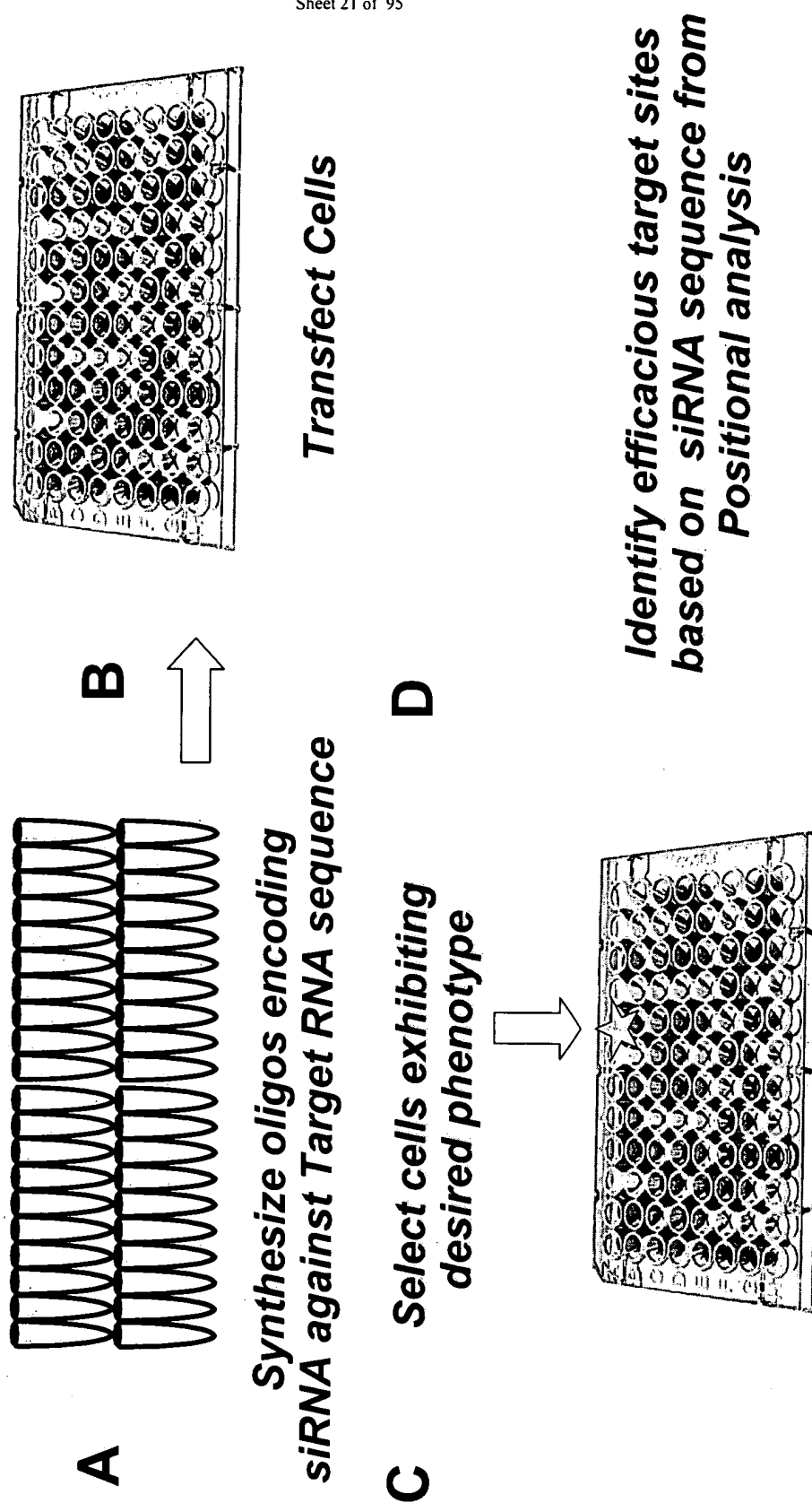
**Figure 19**

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro  
*italic lower case* = 2'-deoxy-2'-fluoro  
underline = 2'-O-methyl

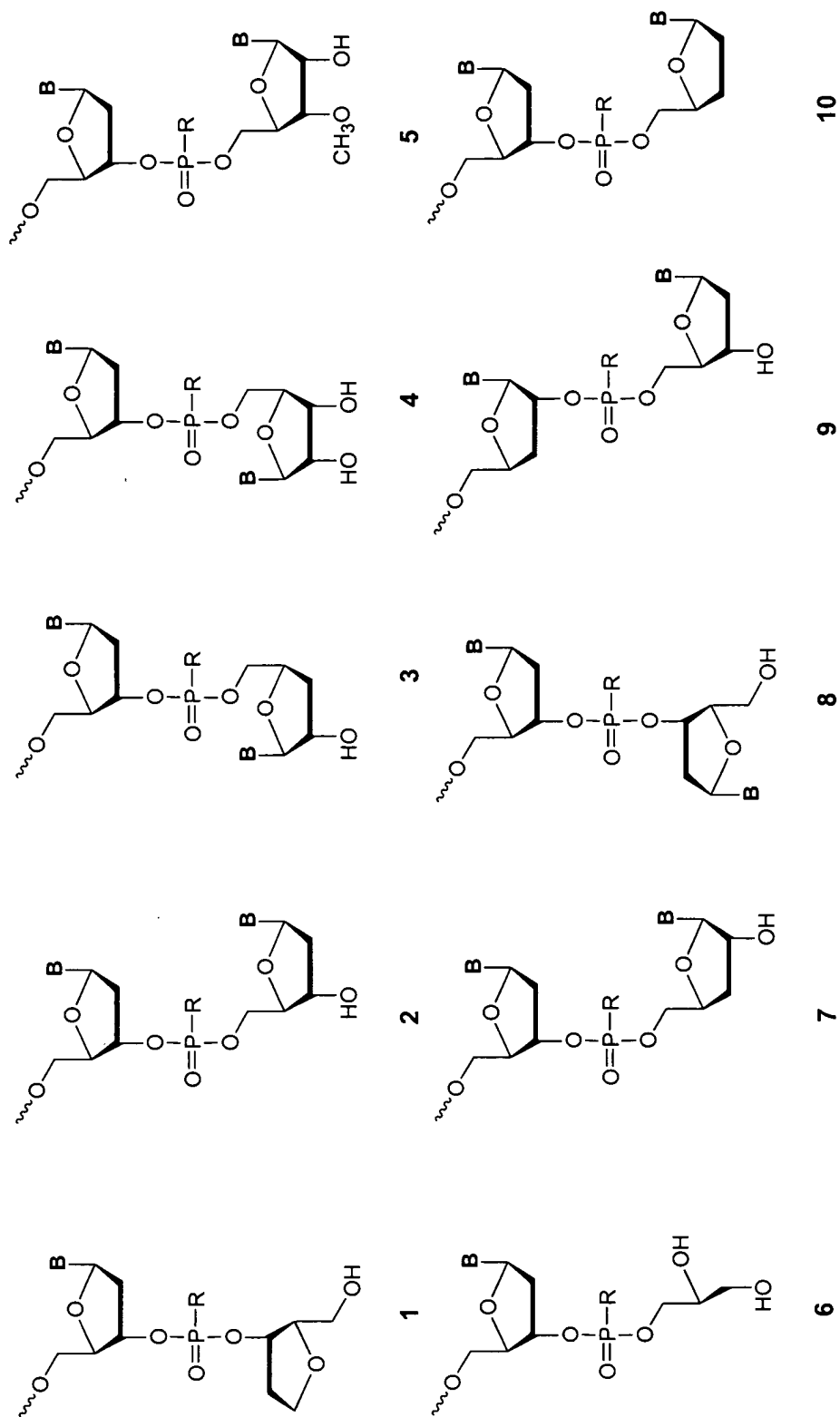
ITALIC UPPER CASE = DEOXY  
 B = INVERTED DEOXYABASIC  
 L = GLYCERYL MOIETY OPTIONALLY PRESENT  
 S = PHOSPHOROTHIOATE OR  
 PHOSPHORODITHIOATE



**Figure 21: Target site Selection using siRNA**

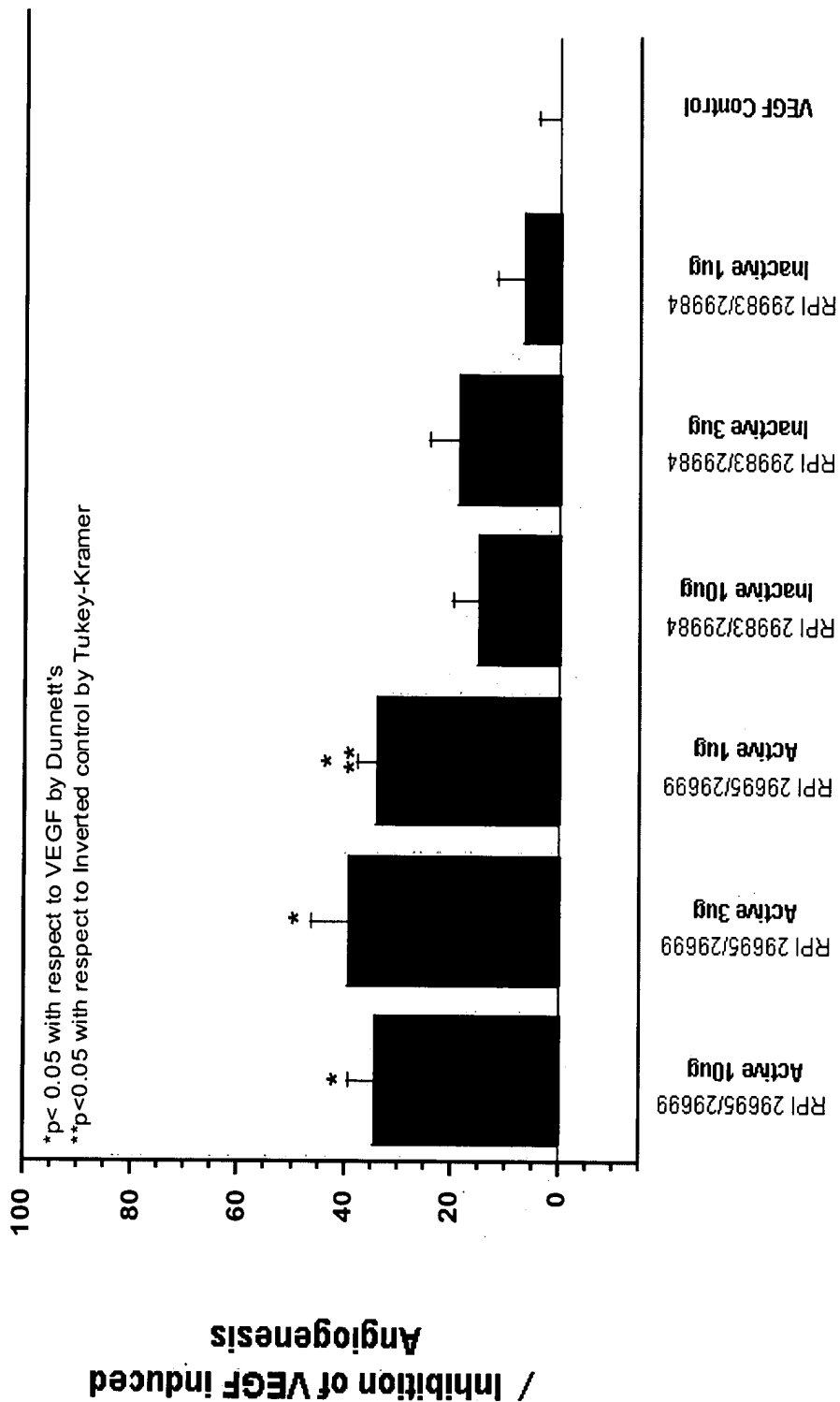


**Figure 22**

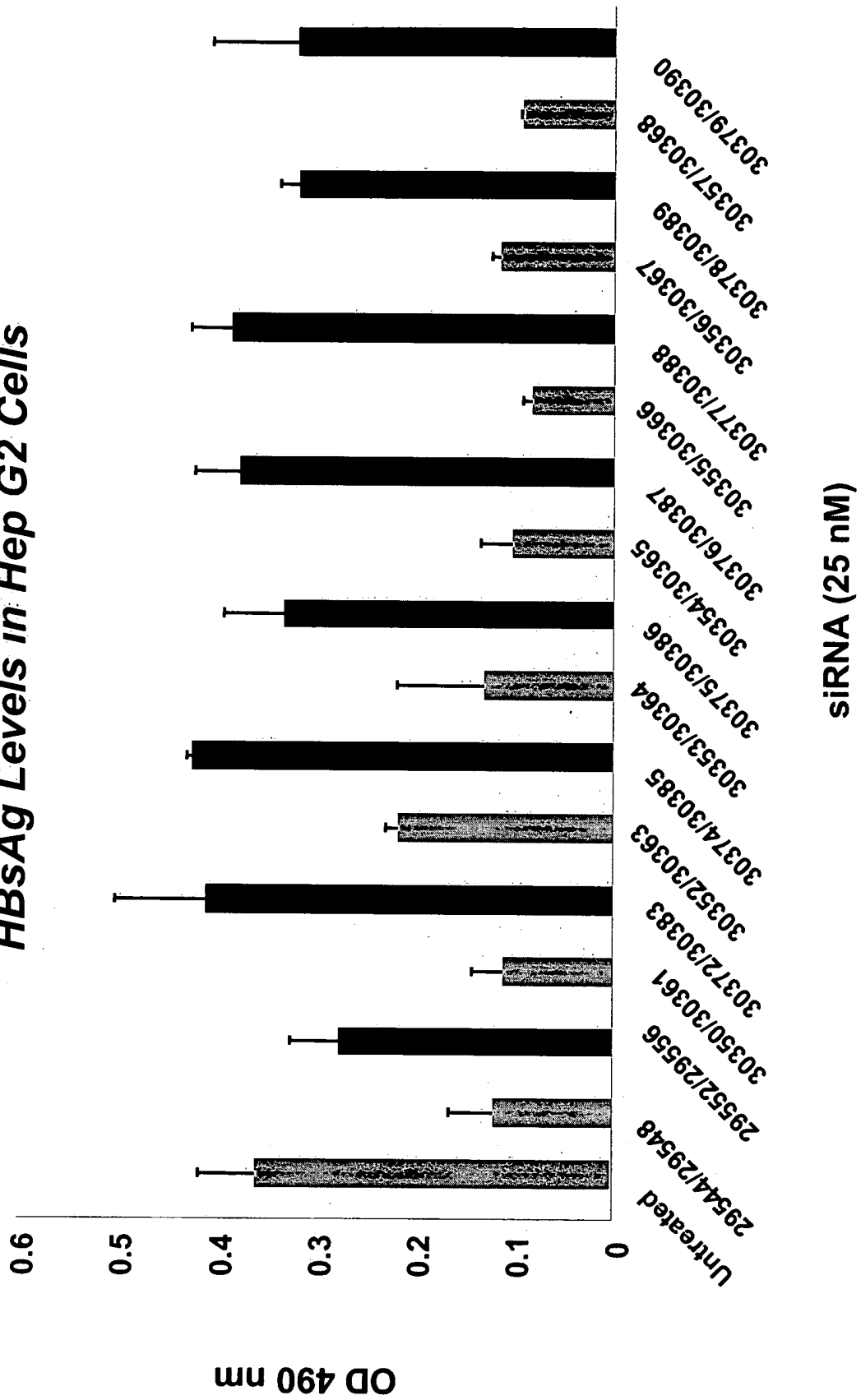


R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl  
 B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

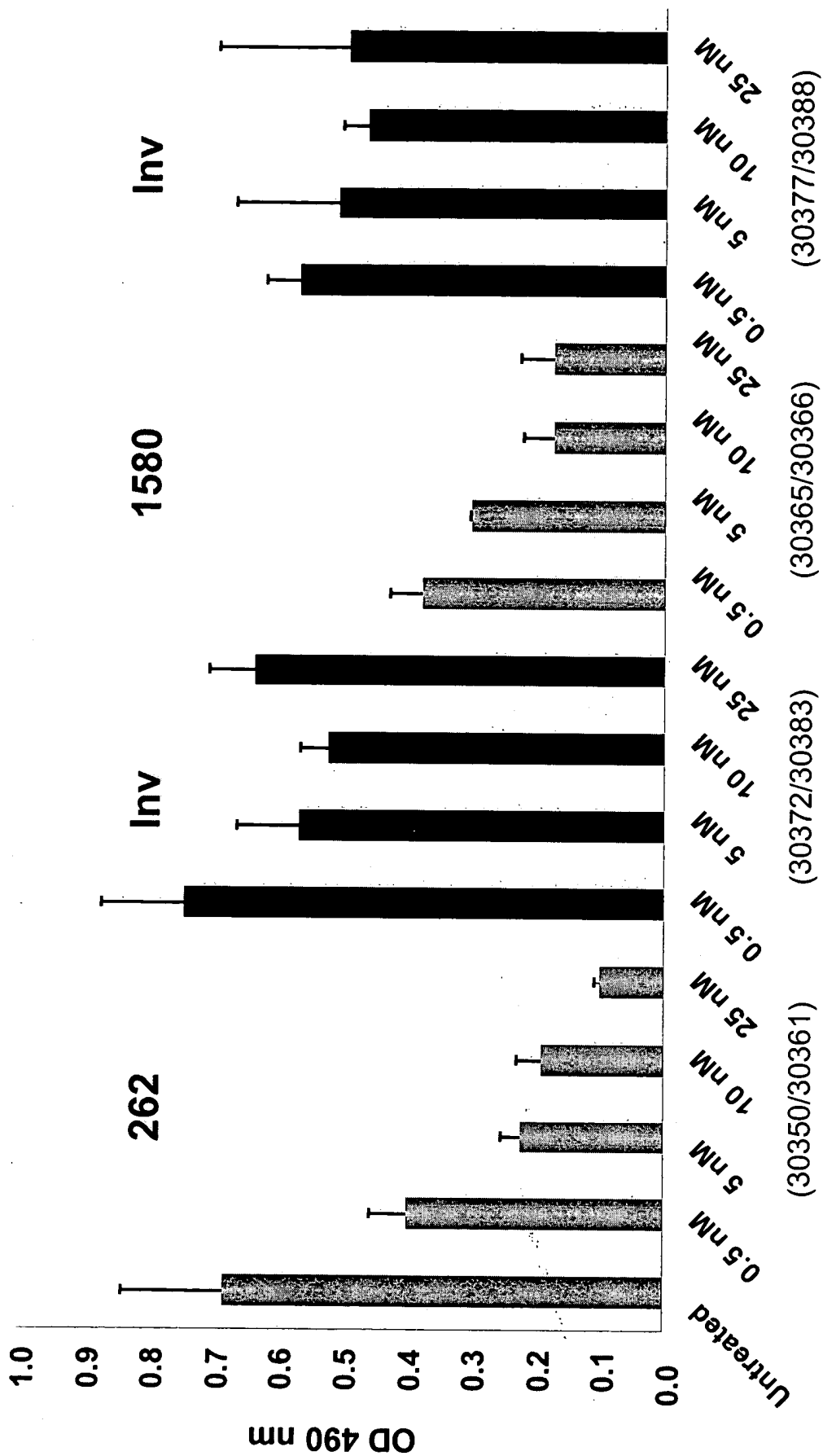
**Figure 23: Inhibition of VEGF-Induced Angiogenesis  
by siRNAs**



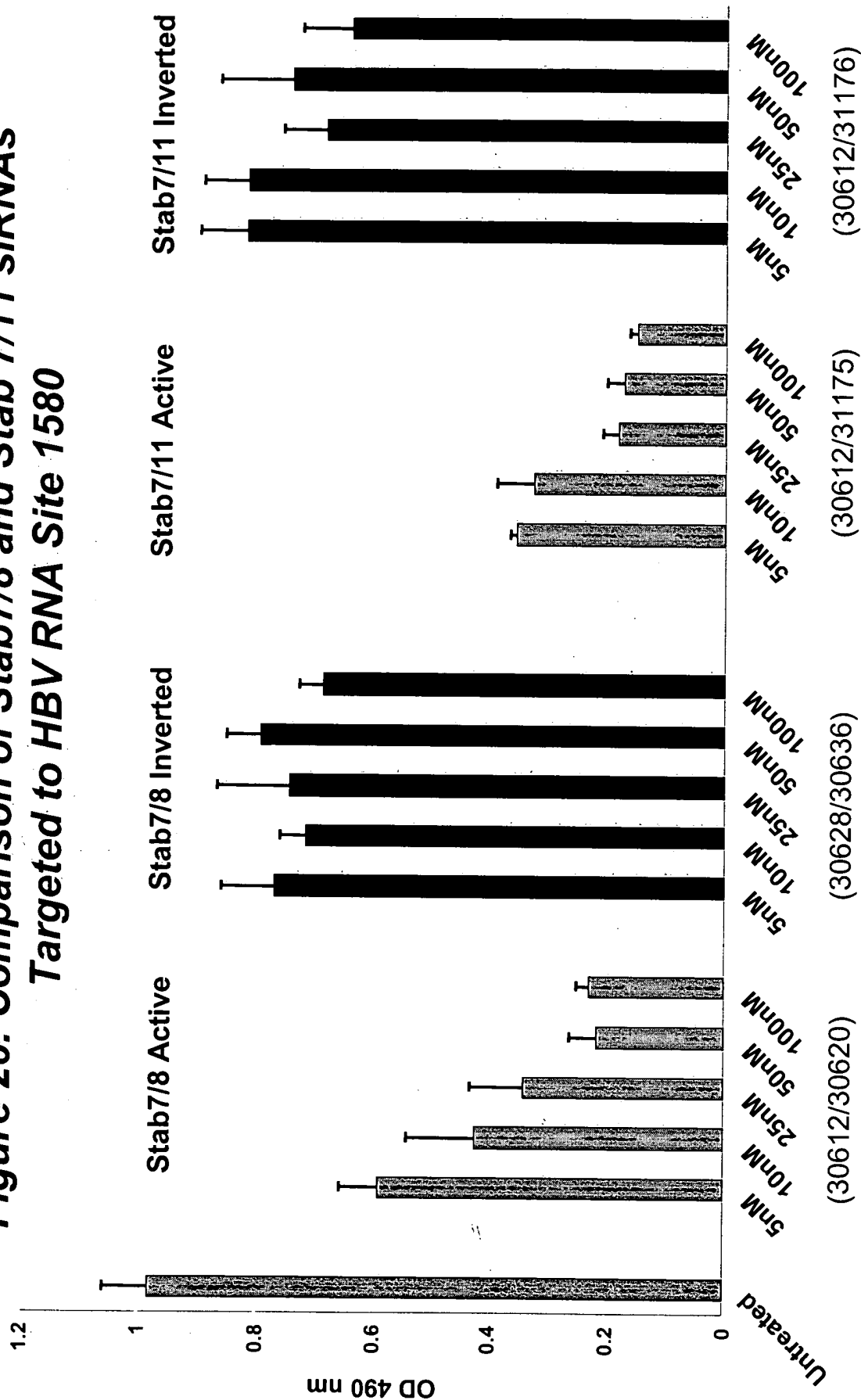
**Figure 24: Stab4/5 siNA Targeted to HBV:  
HBsAg Levels in Hep G2 Cells**



**Figure 25: Dose Response with Stab4/5 siRNAs Targeted to  
 HBV Sites 262 & 1580**



**Figure 26: Comparison of Stab7/8 and Stab 7/11 siRNAs Targeted to HBV RNA Site 1580**



***Figure 27: Modification Strategy***

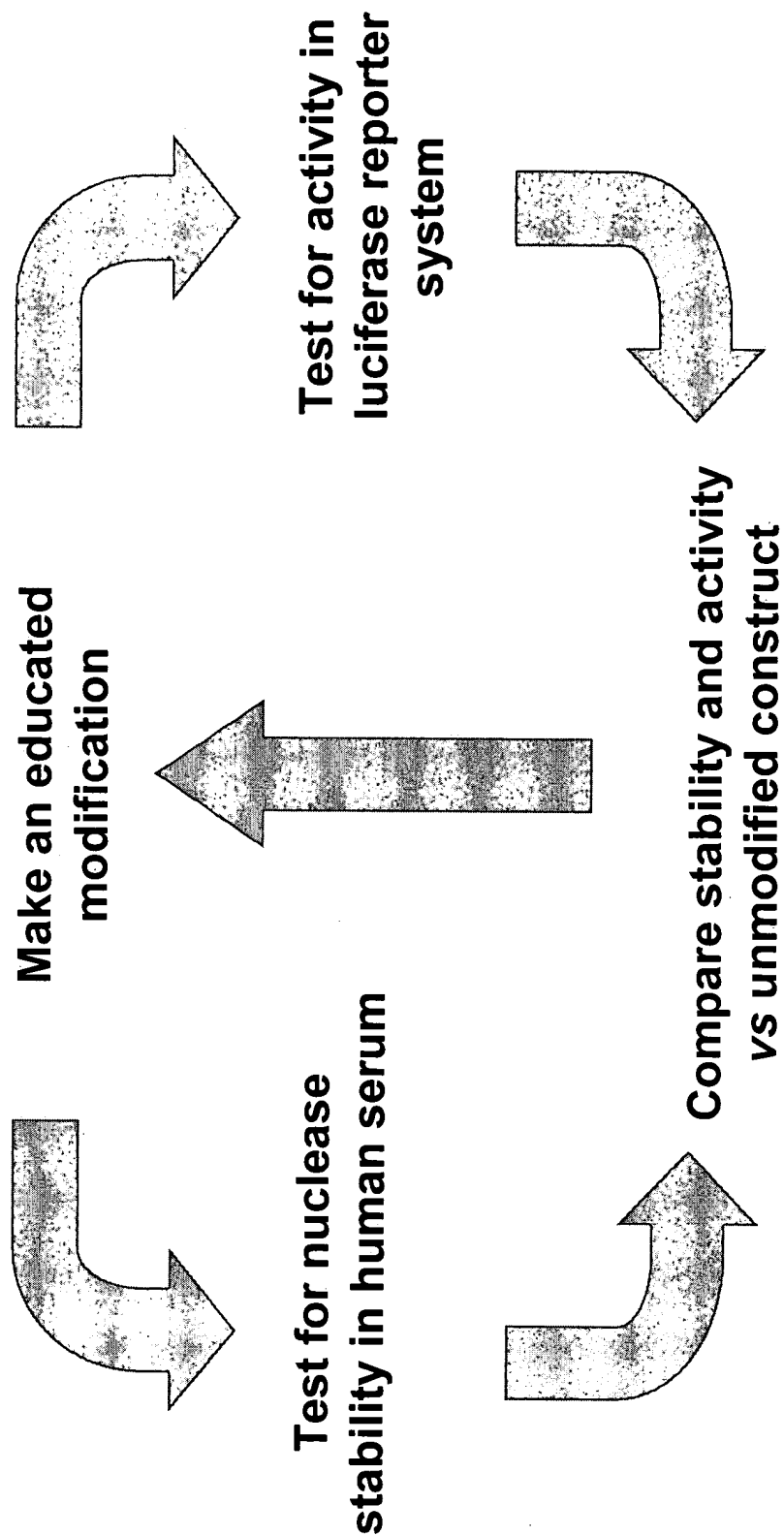


Figure 28: Duration of siRNA Effect  
 All-Ribo vs. Stab4/5 HBV Site 1580: HBsAg Levels

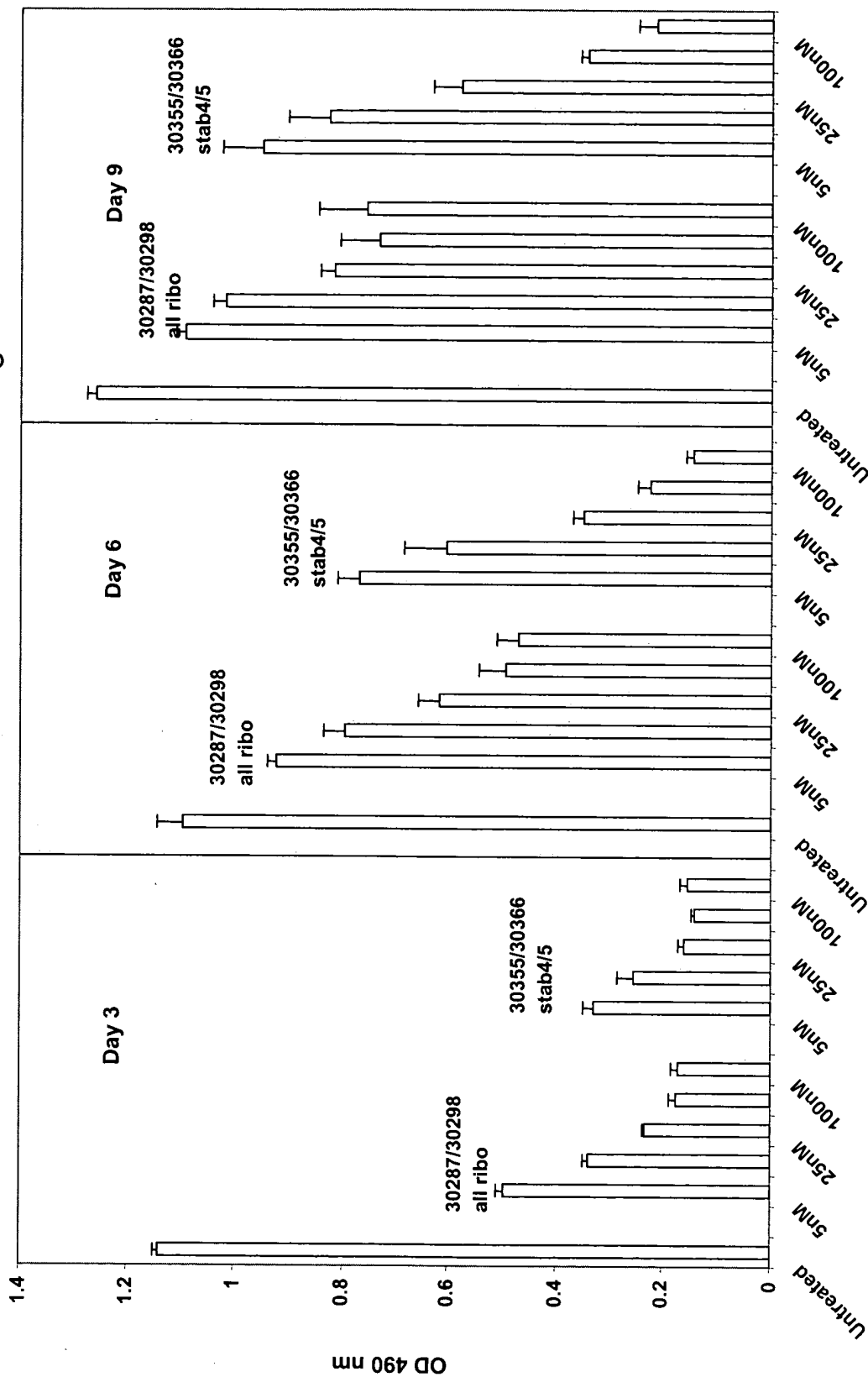


Figure 29: Duration of siRNA Effect  
 All-Ribo vs. Stab7/8 HBV Site 1580: HBsAg Levels

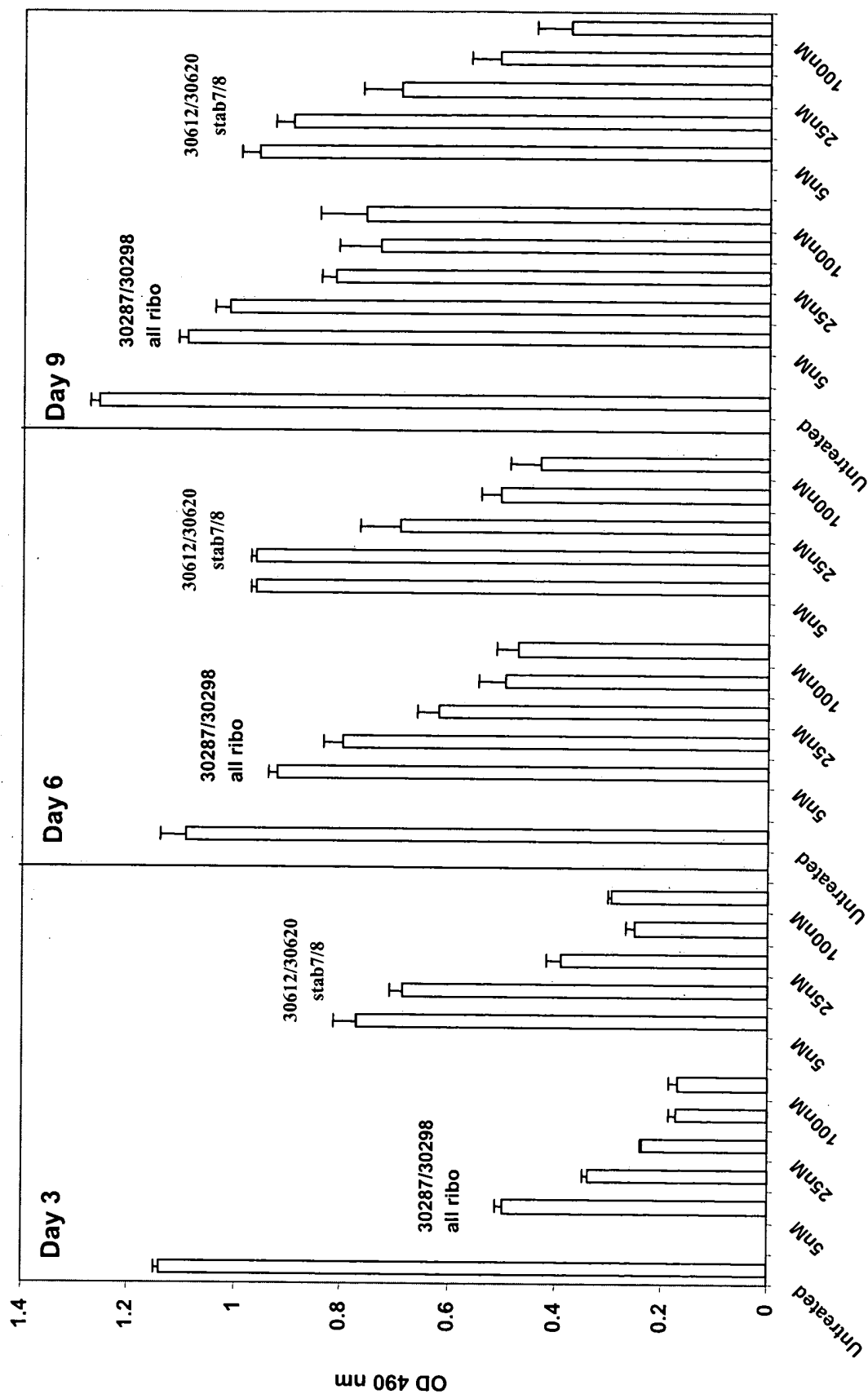


Figure 30: Duration of siRNA Effect  
 All-Ribo vs. Stab7/11 HBV Site 1580: HBsAg Levels

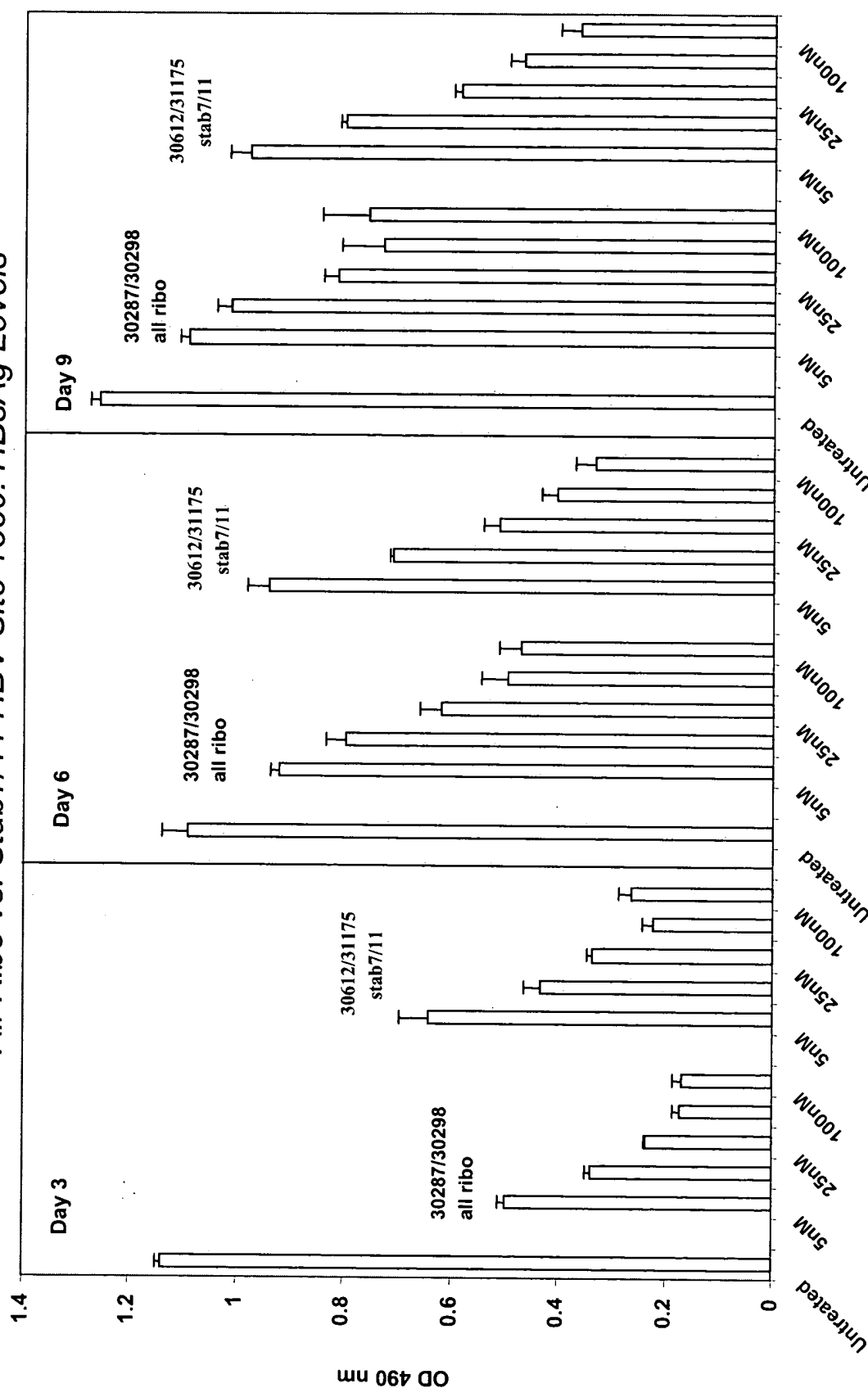
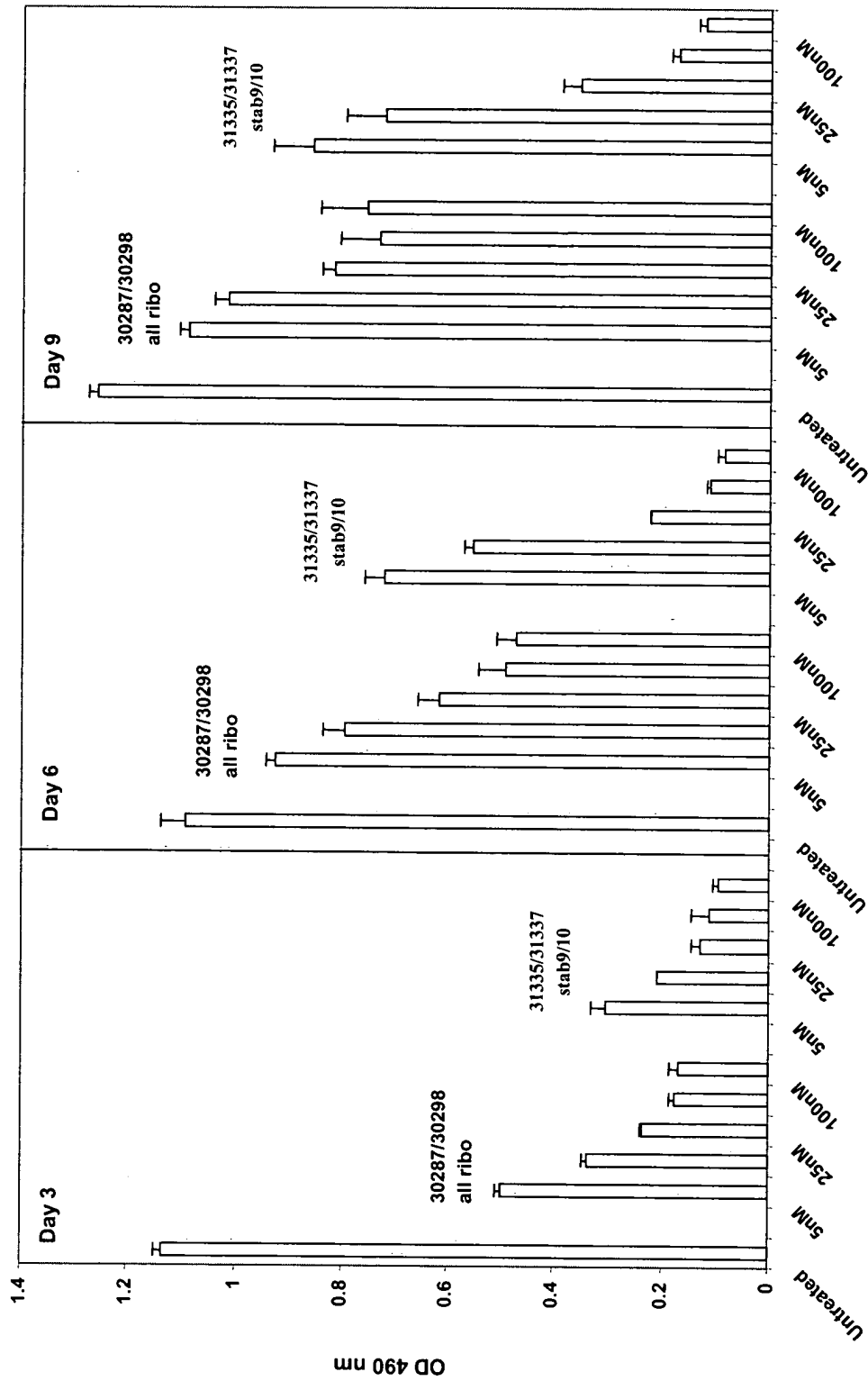
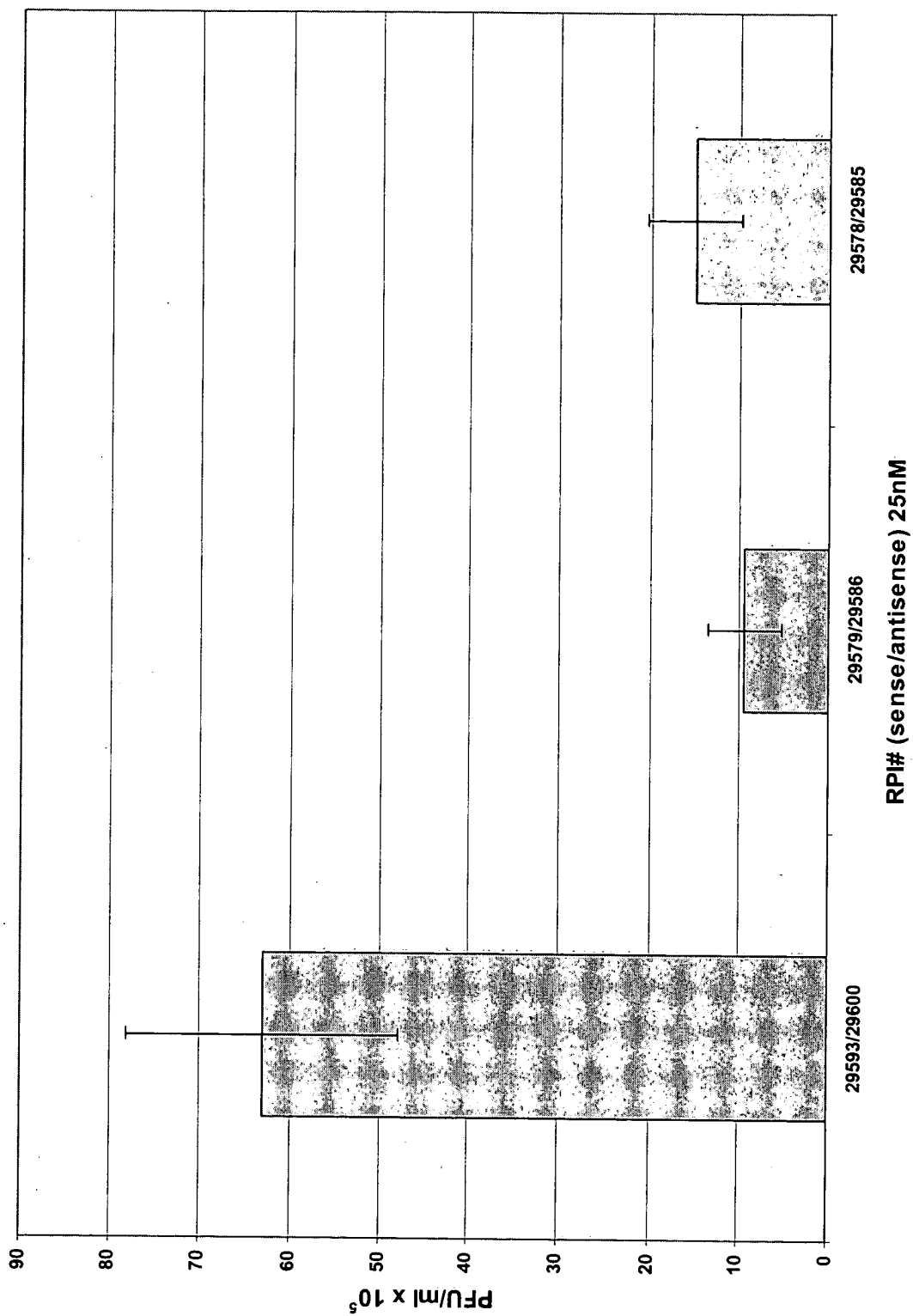


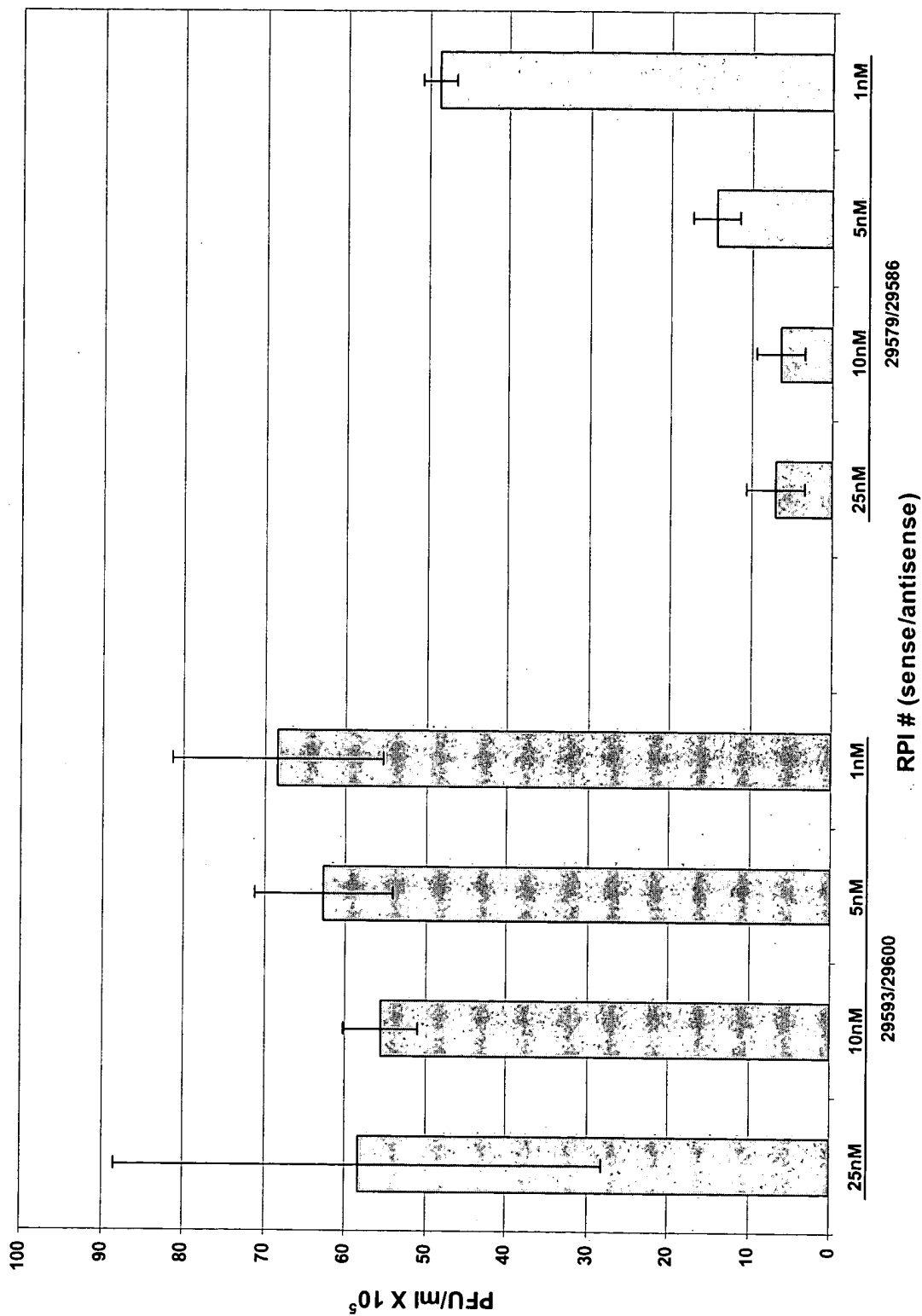
Figure 31: Duration of siRNA Effect  
 All-Ribo vs. Stab9/10 HBV Site 1580: HBsAg Levels



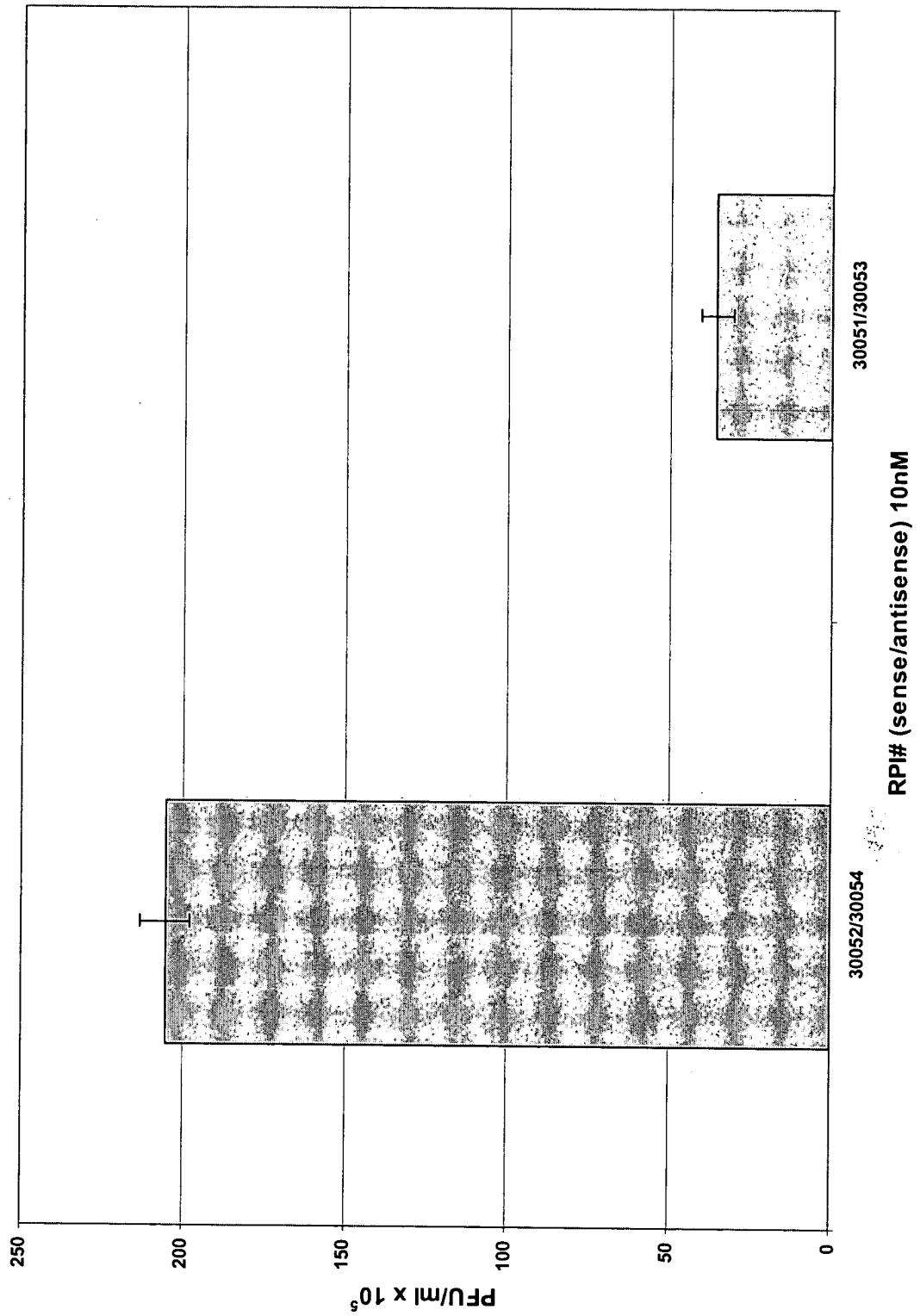
**Figure 32 : siRNAs targeting HCV chimera**



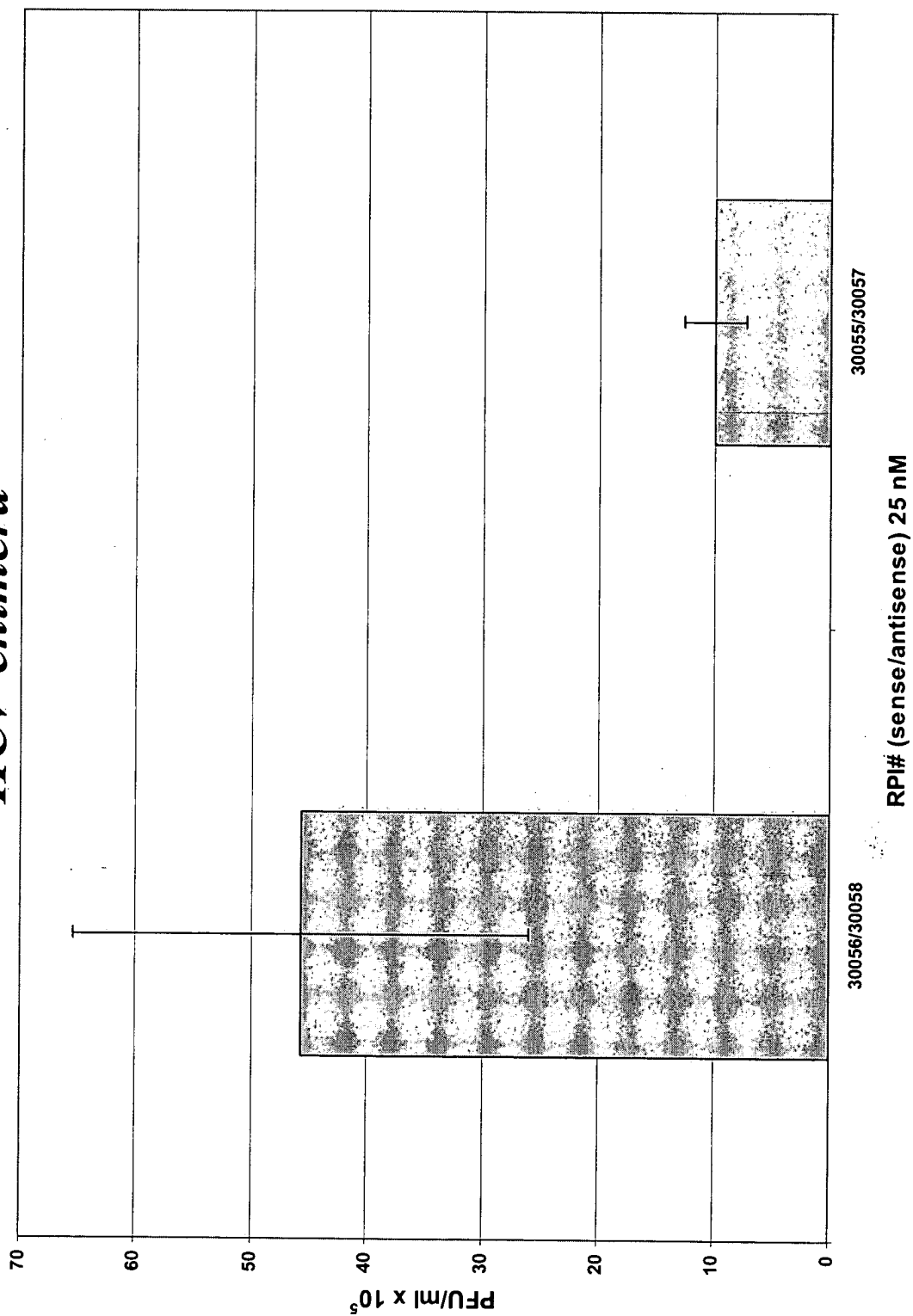
**Figure 33: HCV siRNA dose response**



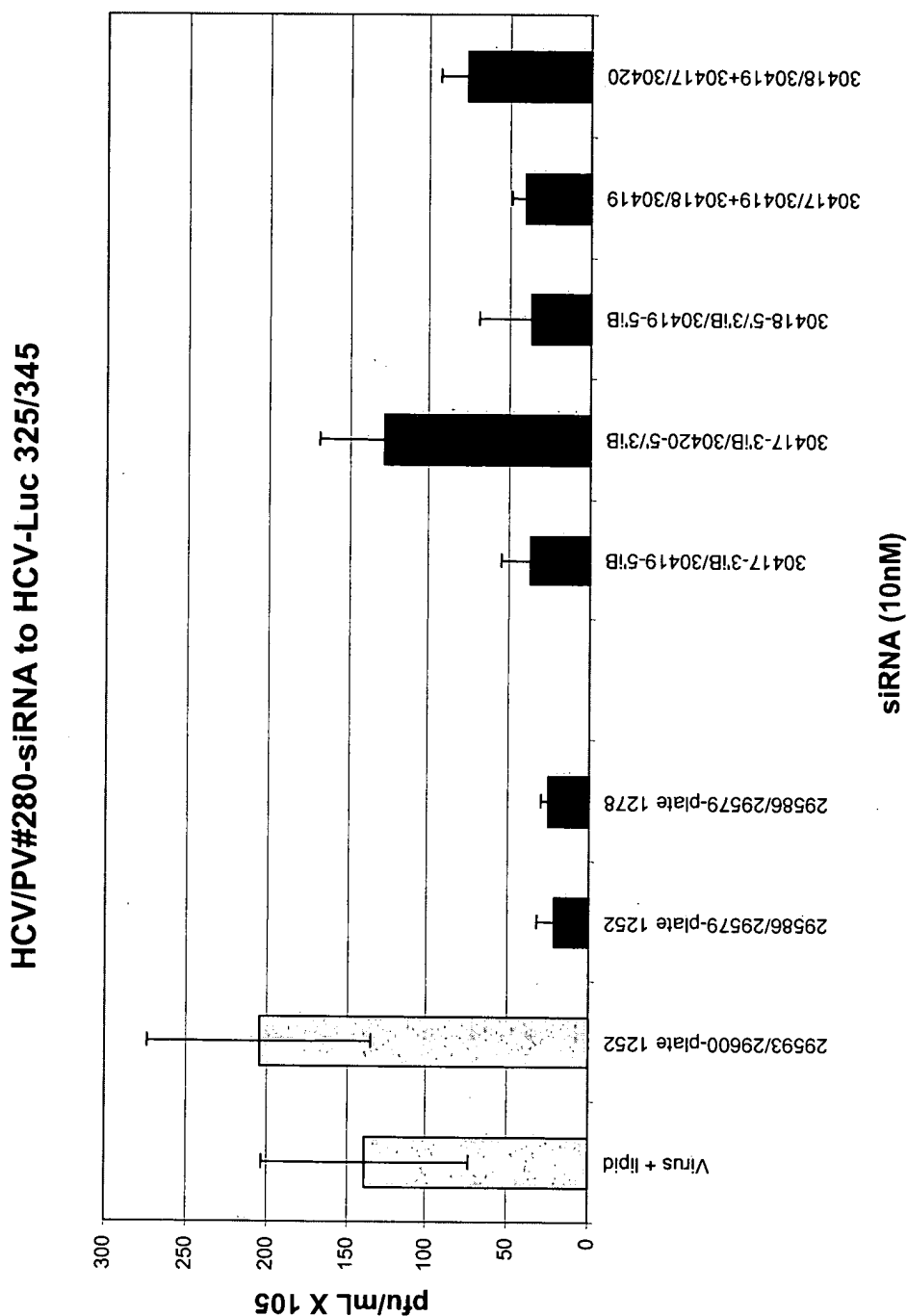
**Figure 34: Chemically Modified siRNA targeting  
HCV chimera**



**Figure 35: Chemically Modified siRNA targeting  
HCV chimera**

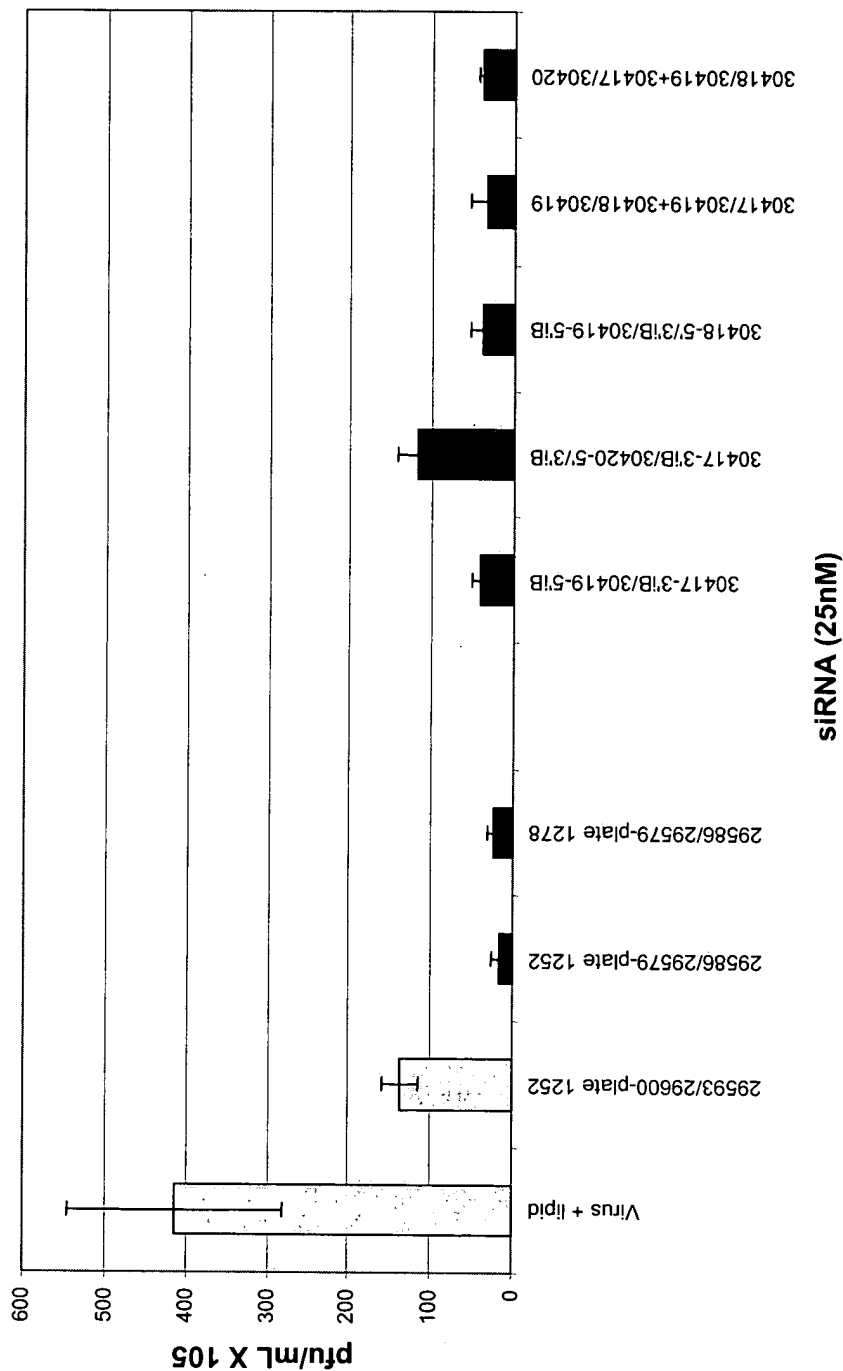


**Figure 36: Chemically Modified siRNA  
 targeting HCV chimera**

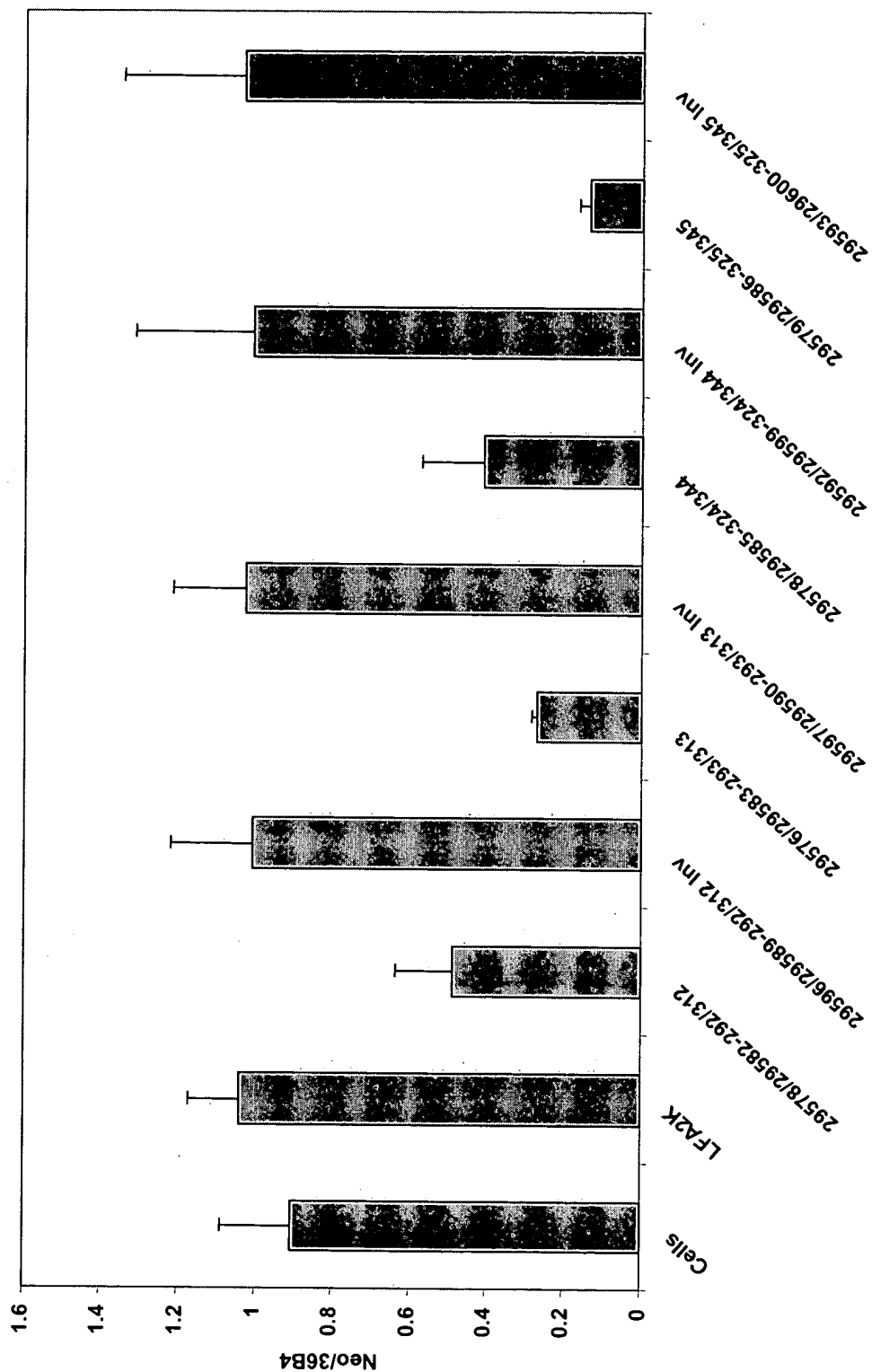


**Figure 37: Chemically Modified siRNA  
 targeting HCV chimera**

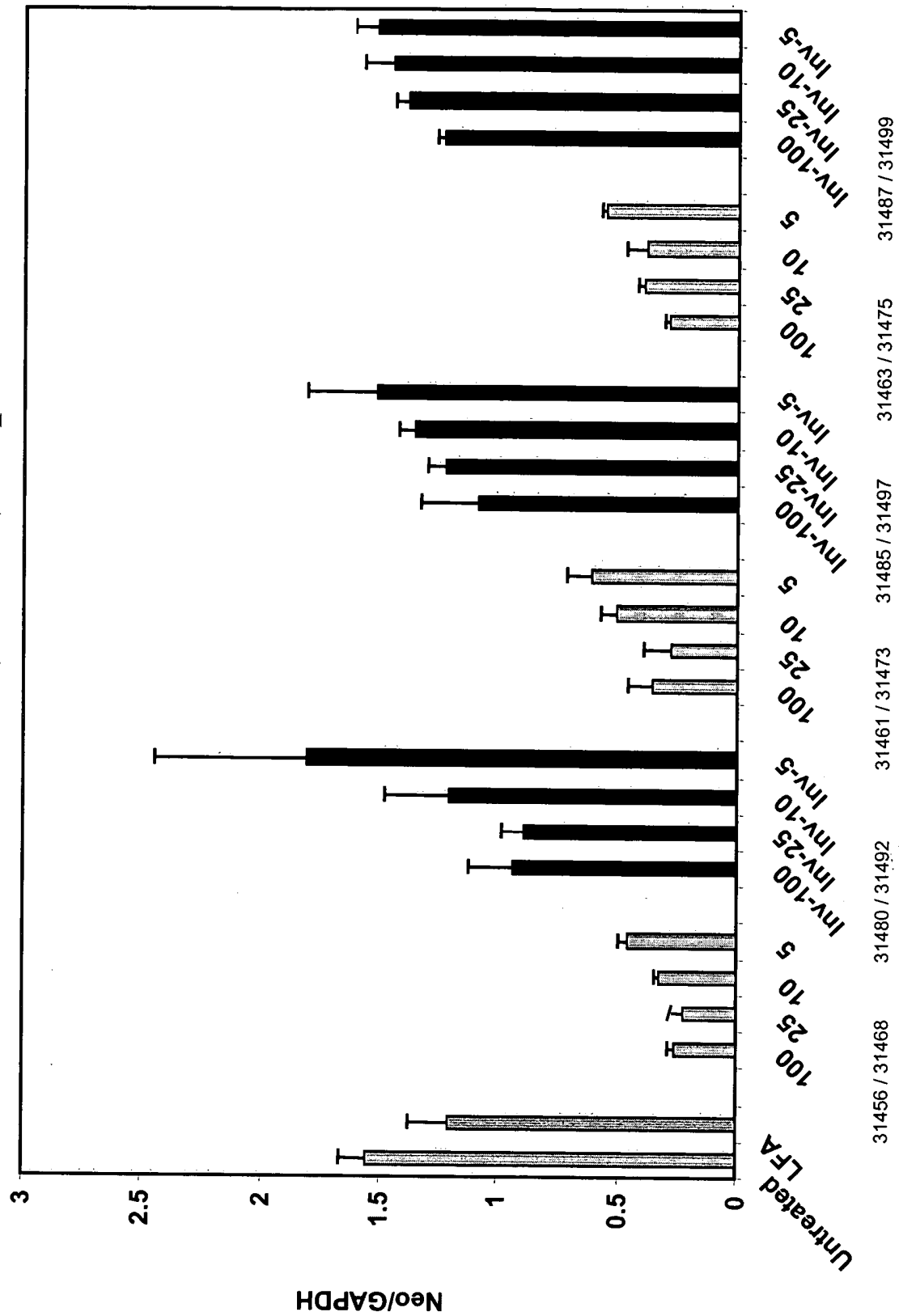
HCV/PV#280-siRNA to HCV-Luc site 325/345



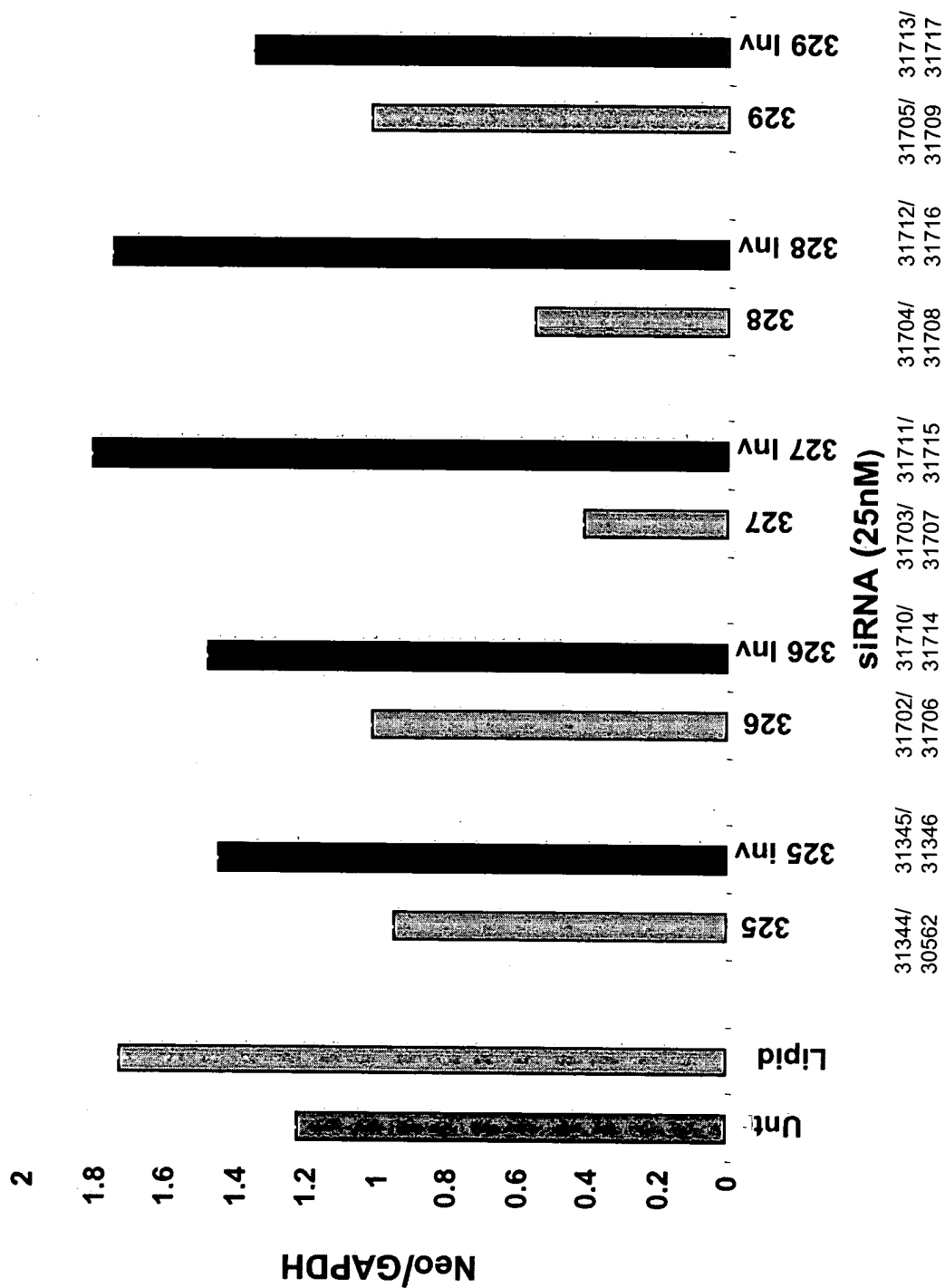
**Figure 38: HCV/Replicon Cells transfected  
 with 0.5µl/well LFA 2K-72 hours**



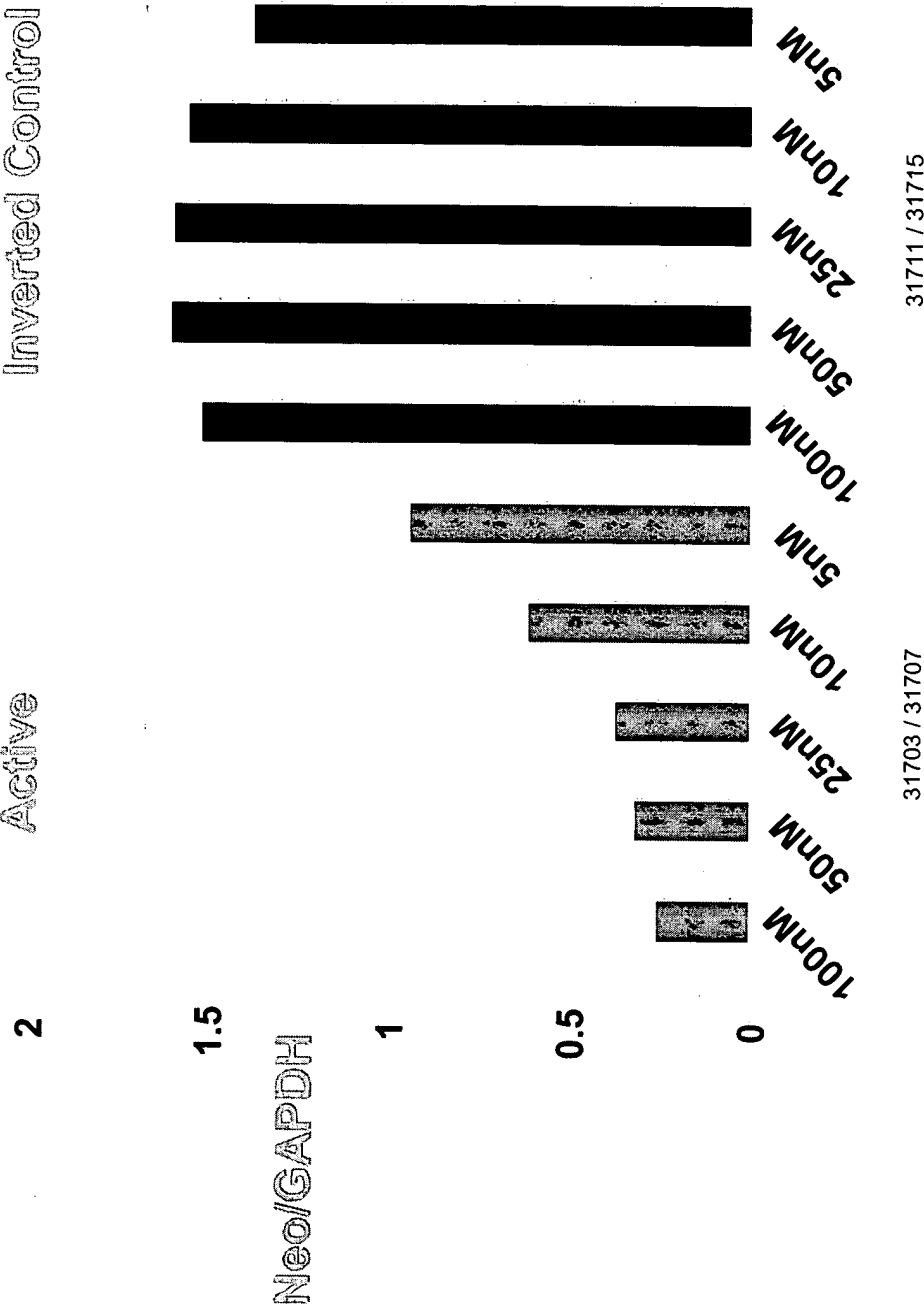
**Figure 39: Dose Response with Stab4/5 siNA Leads  
 in HCV Subgenomic Replicon**



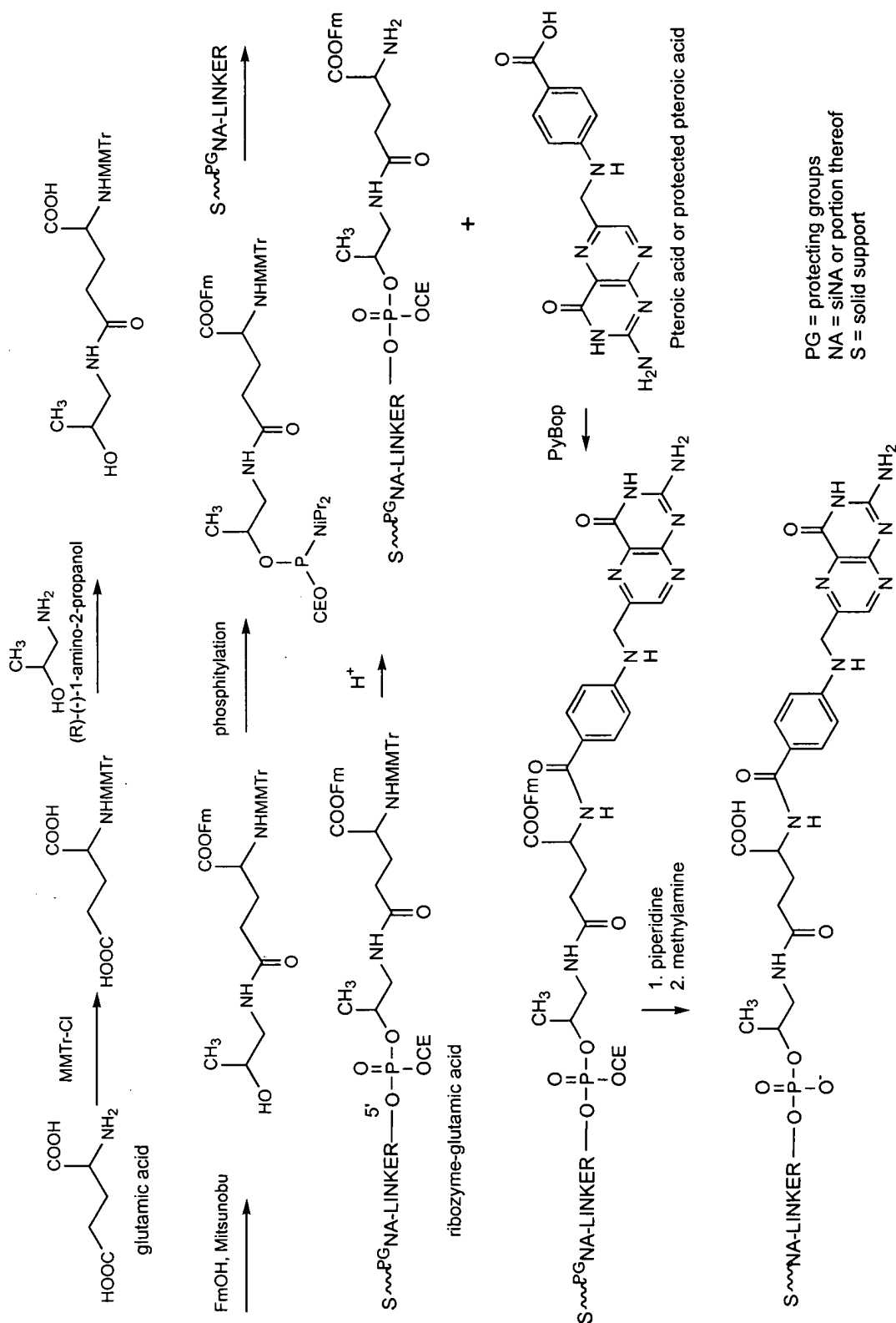
**Figure 40: Activity of Stab 7/8 siNA Leads in HCV Subgenomic Replicon**



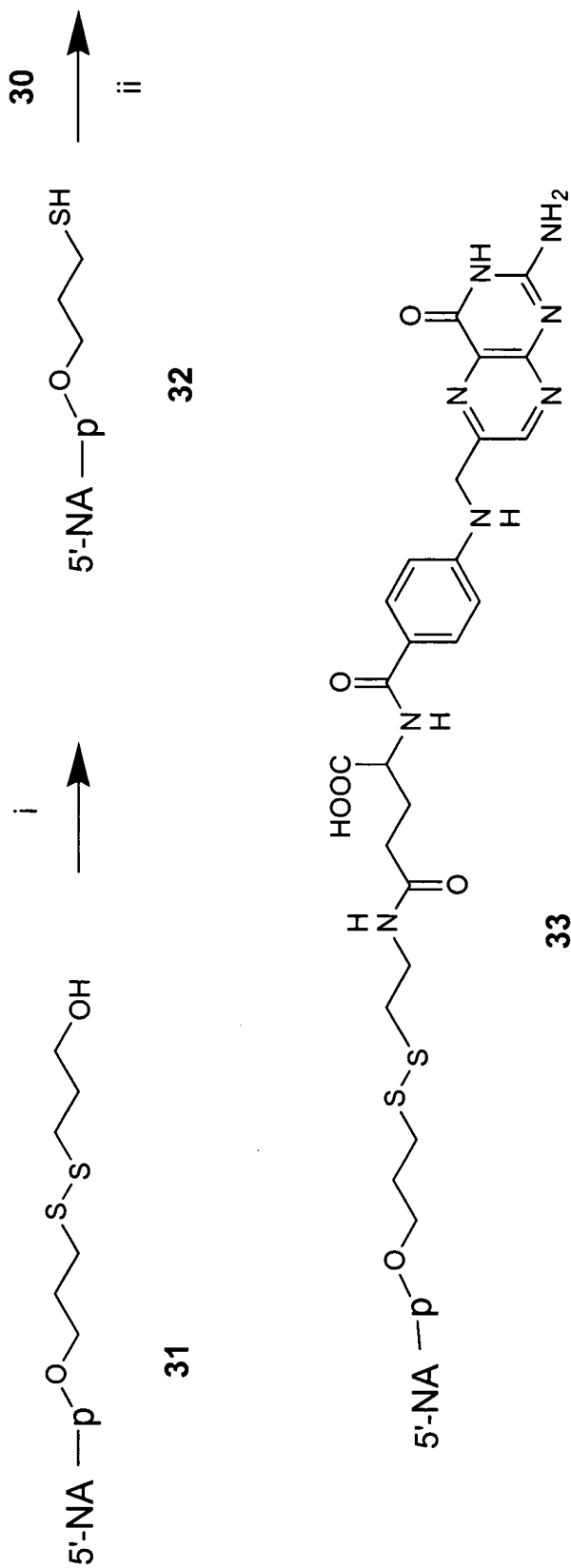
*Figure 41: Dose Response with Fully Modified  
HCV Site 327 siNA*



**Figure 42: Solid Phase Post-synthetic conjugation of pterioic acid**

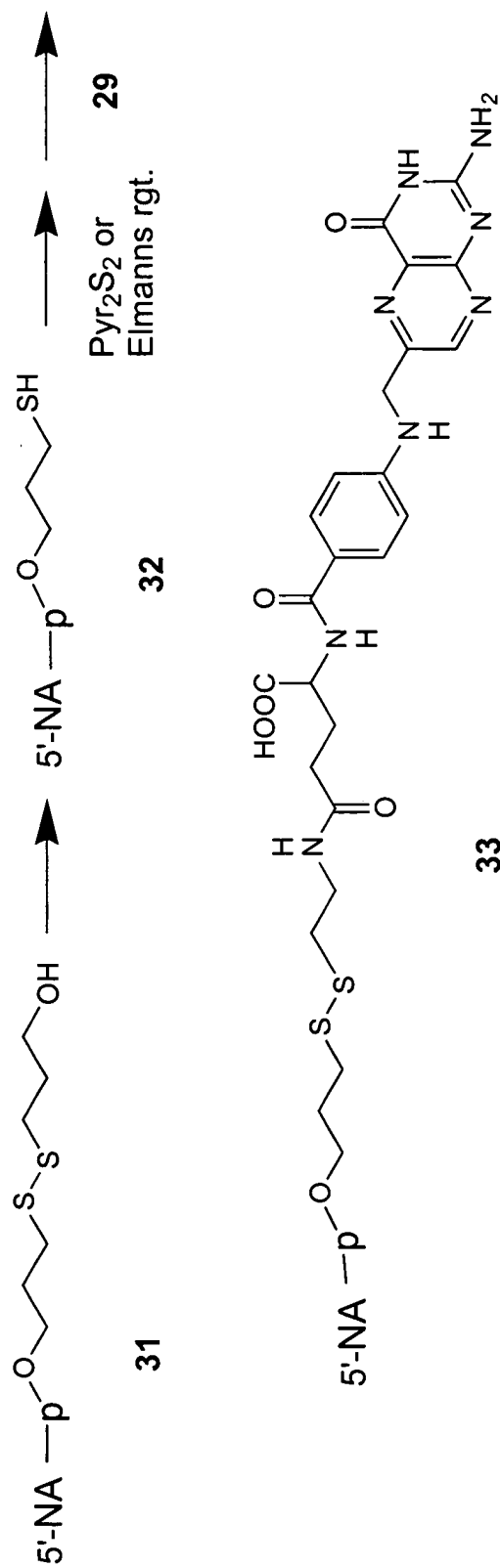


**Figure 43**

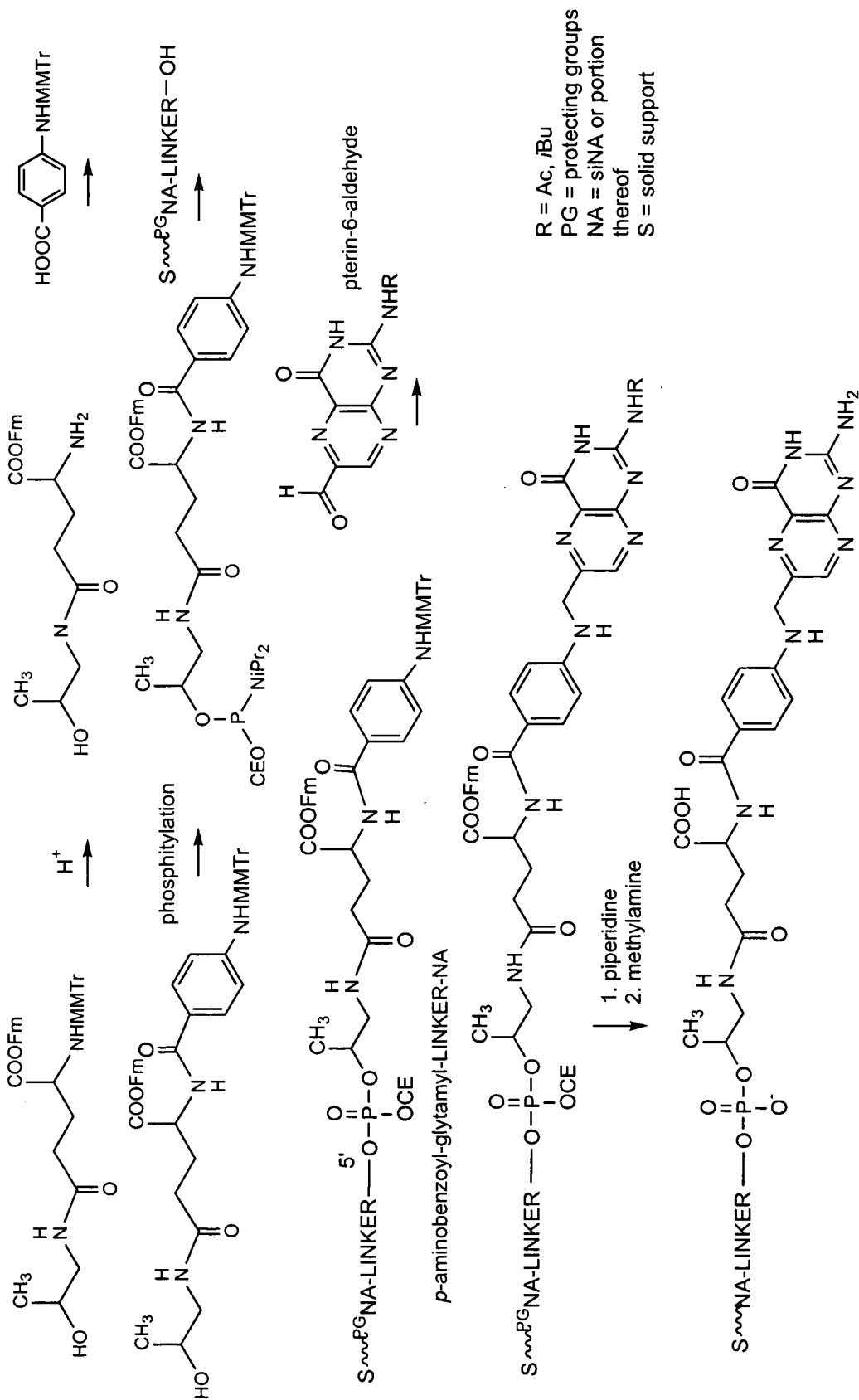


NA = siNA or a portion thereof  
 p = phosphorous moiety

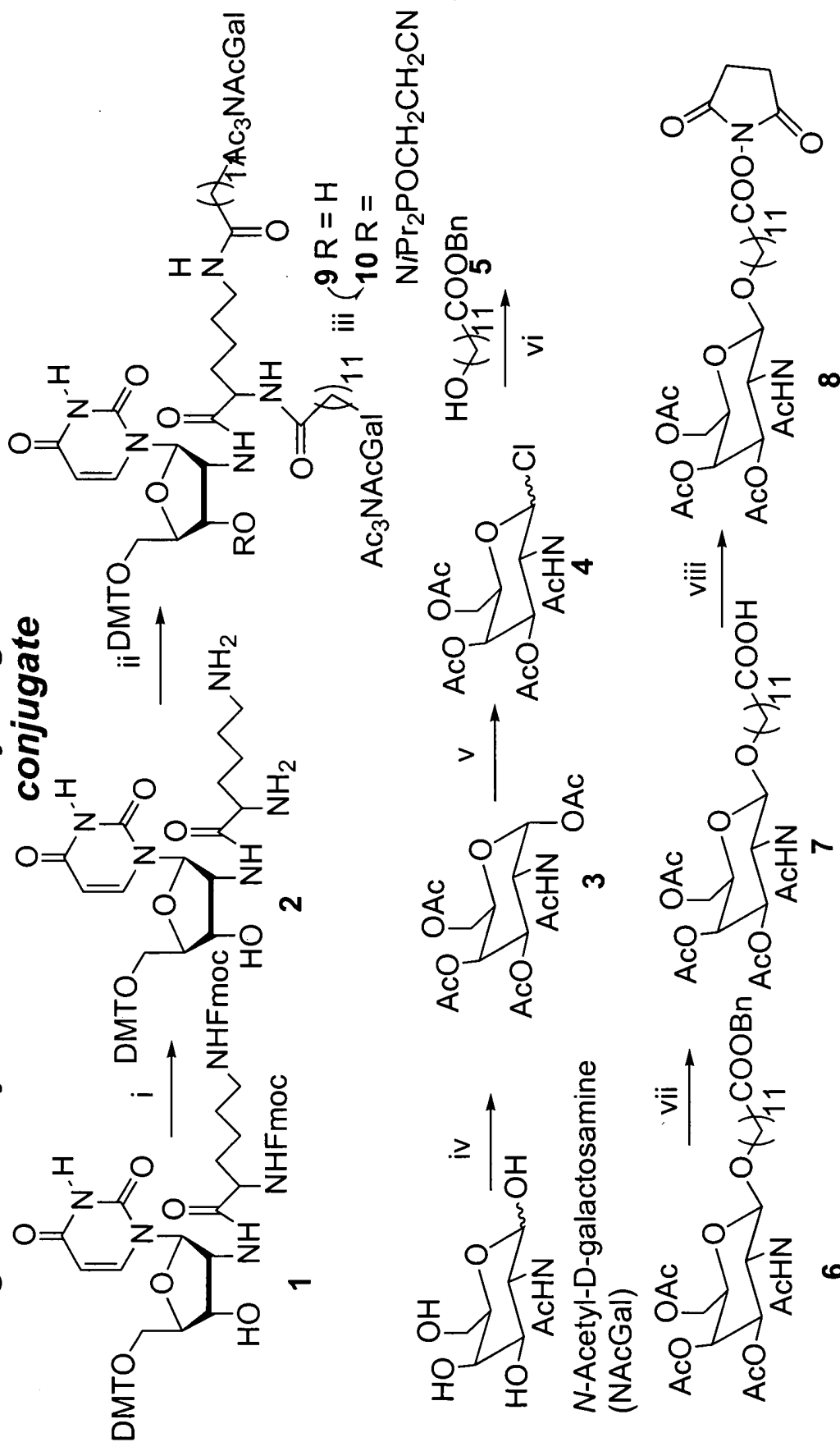
**Figure 44**



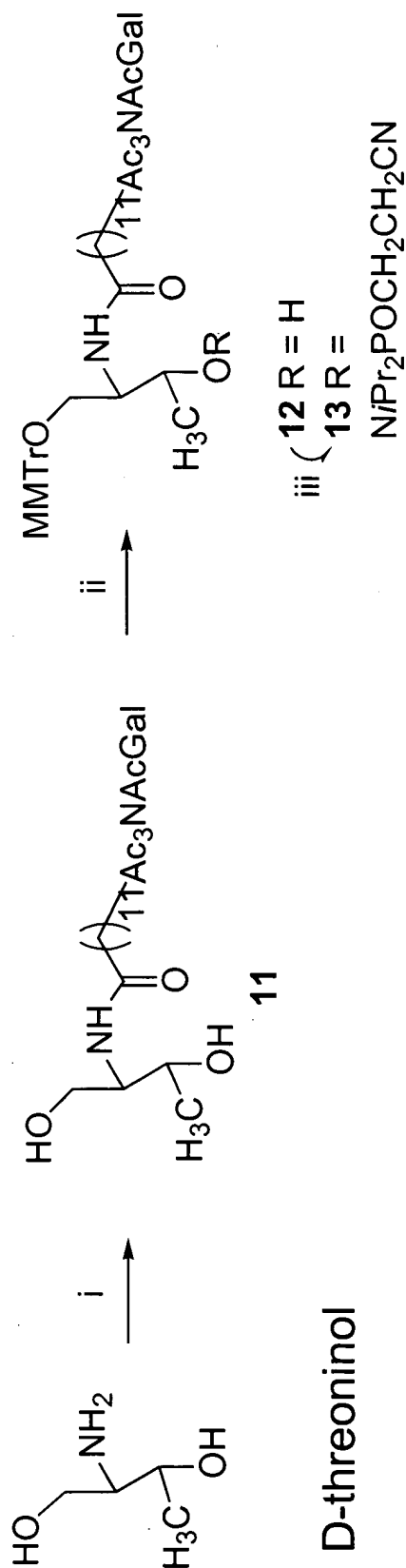
NA = siNA or a portion thereof  
 p = phosphorous moiety

**Figure 45: Solid Phase Post-synthetic conjugation of pterioic acid**

**Figure 46: Synthesis of N-acetyl-D-galactosamine-2'-aminouridine**

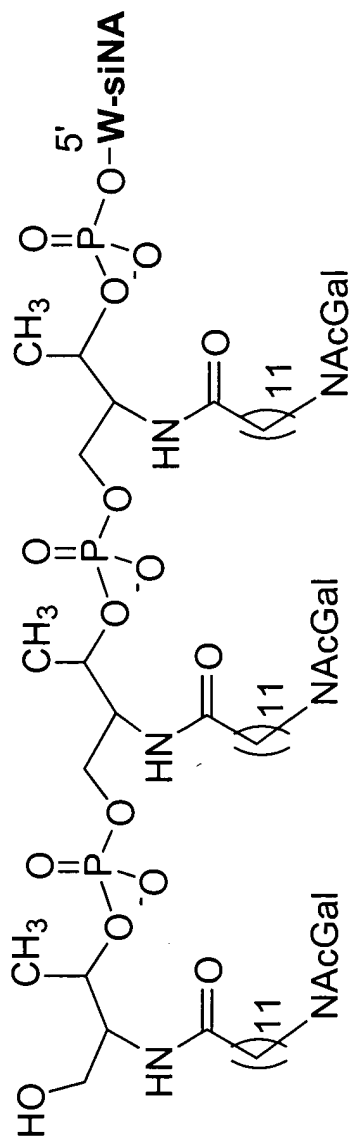


**Figure 47: Synthesis of *N*-acetyl-*D*-galactosamine-*D*-threoninol conjugate**



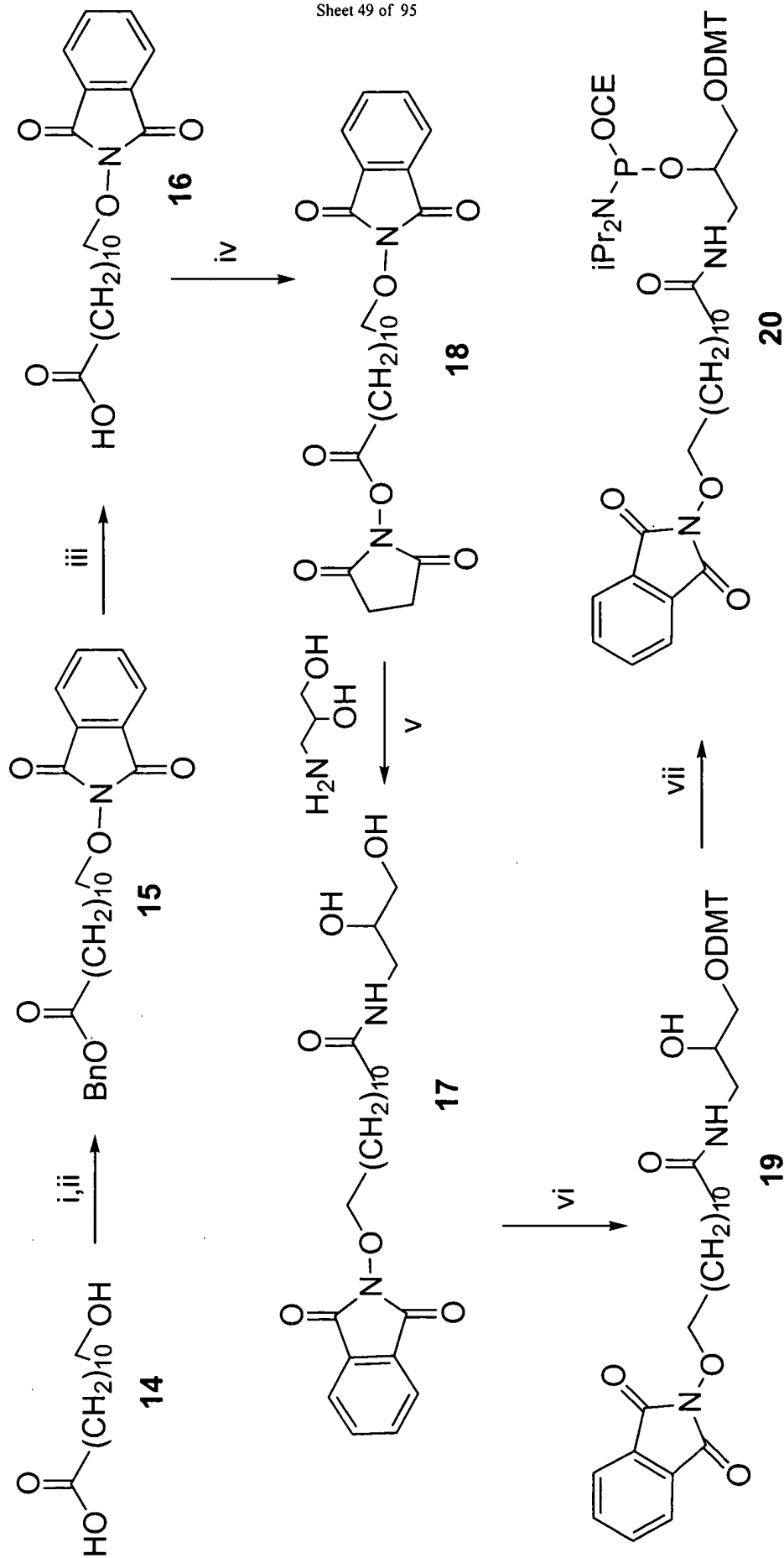
Reagents and Conditions: (i) 7, DCC, *N*-hydroxysuccinimide, (ii) MMTTr-Cl, pyridine, (iii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>.

**Figure 48: Conjugation of targeting ligands to the 5'-end of a siNA molecule**

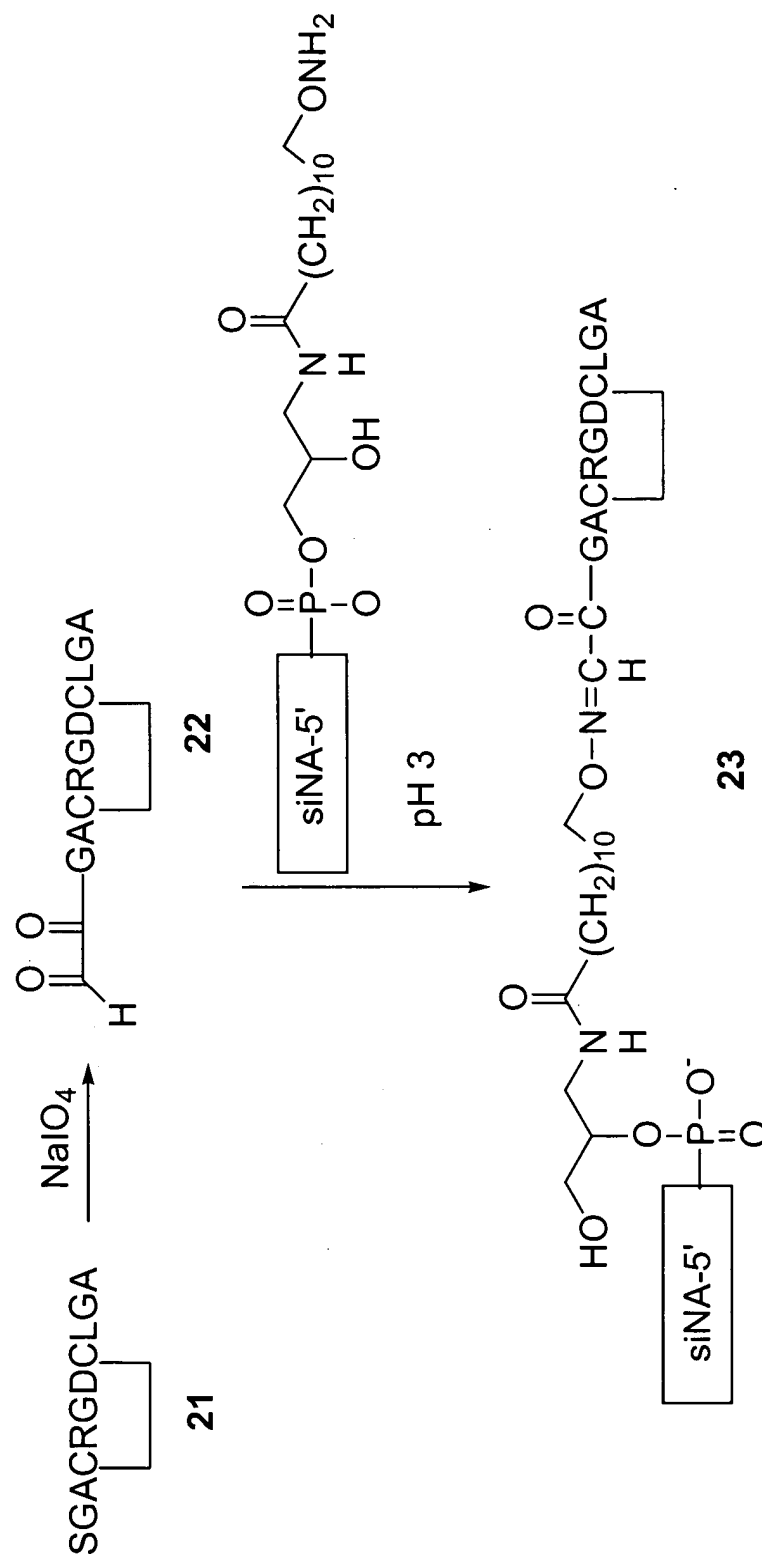


**N-acetyl-D-galactosamine conjugate**

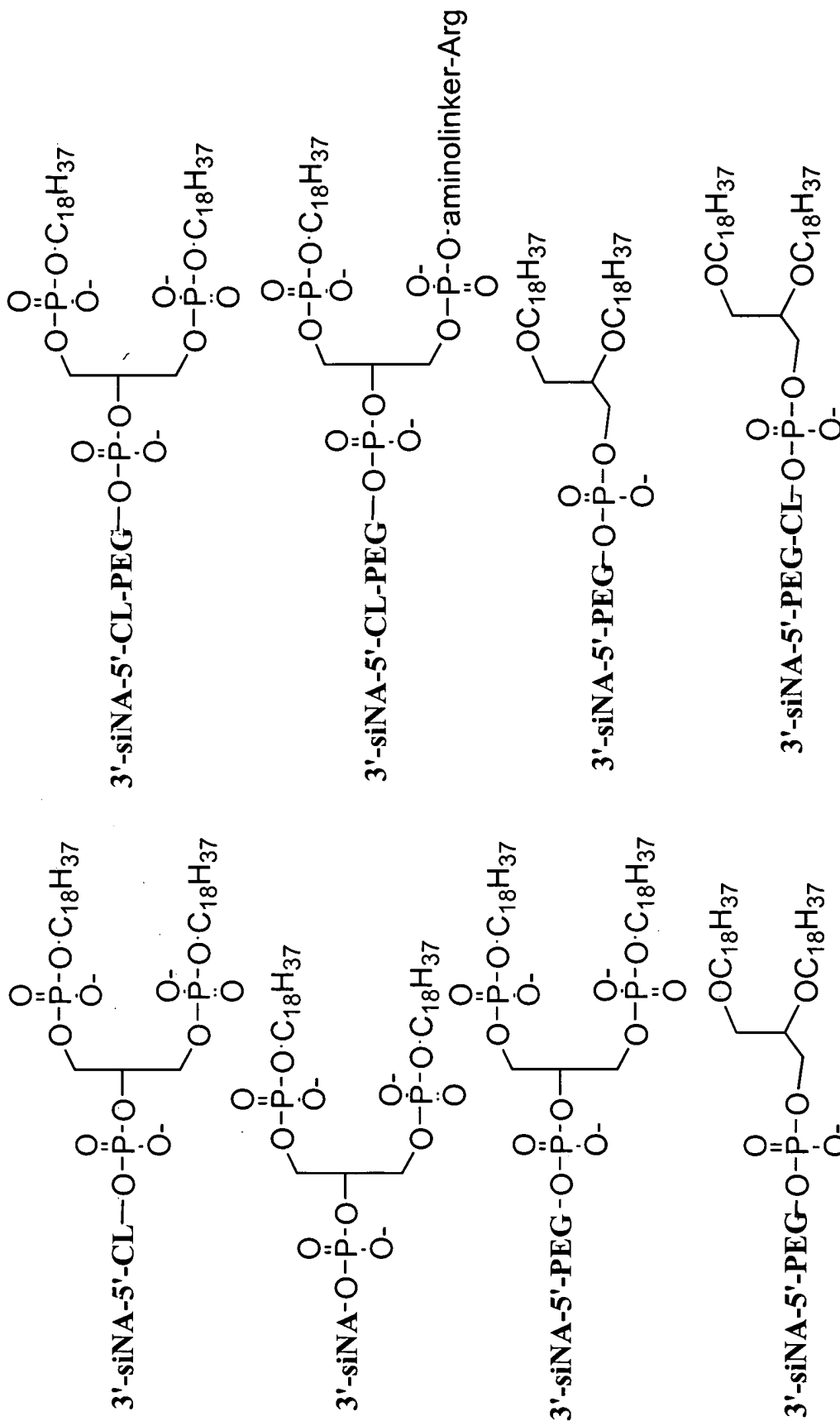
**Figure 49: Synthesis of dodecanoic acid linker**



**Figure 50: Oxime linked siNA/Peptide Conjugate**



**Figure 51: siNA/Phospholipid Conjugates**

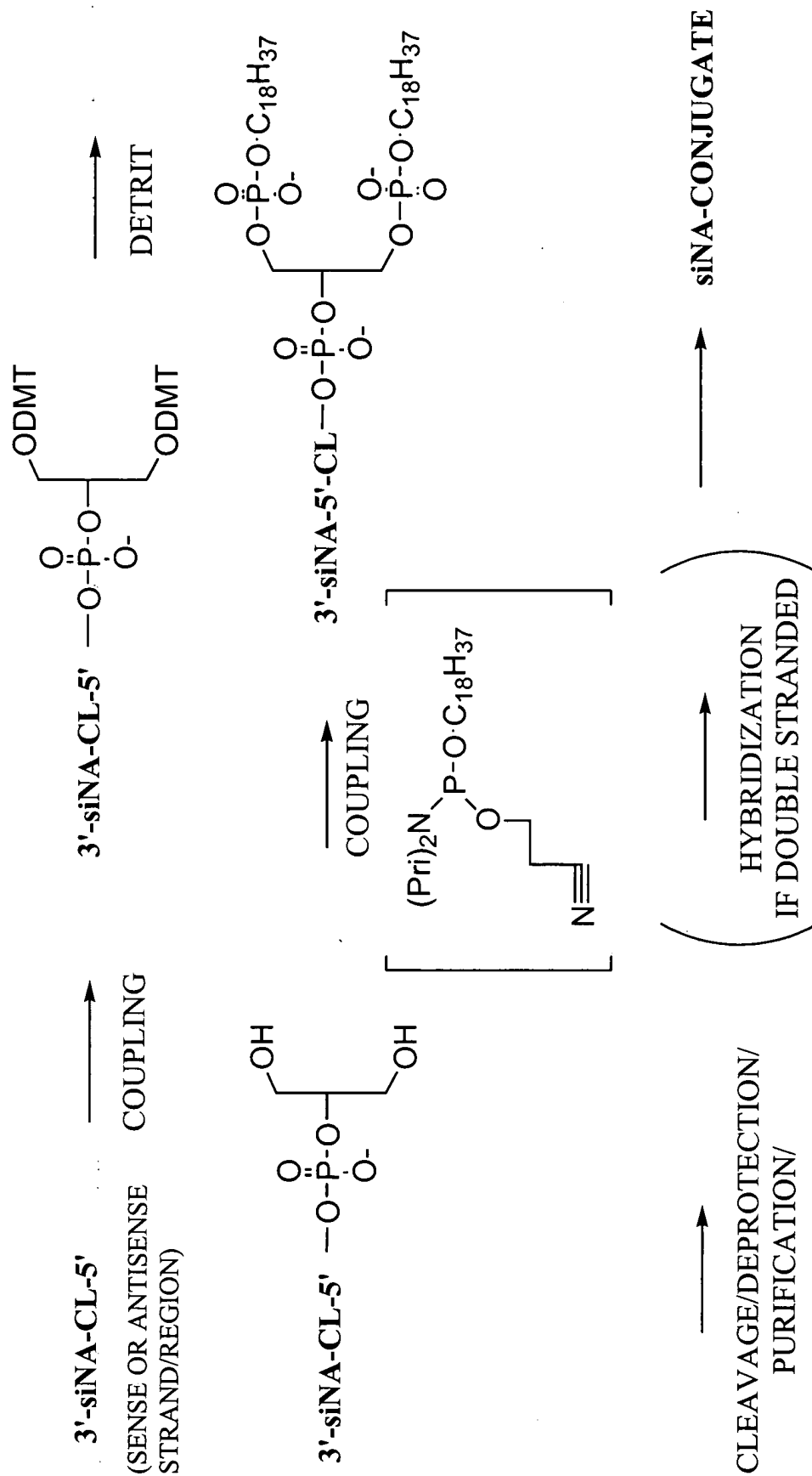


PEG=polyethylene glycol

CL=cleavable linker (e.g. A-dT, C-dT)

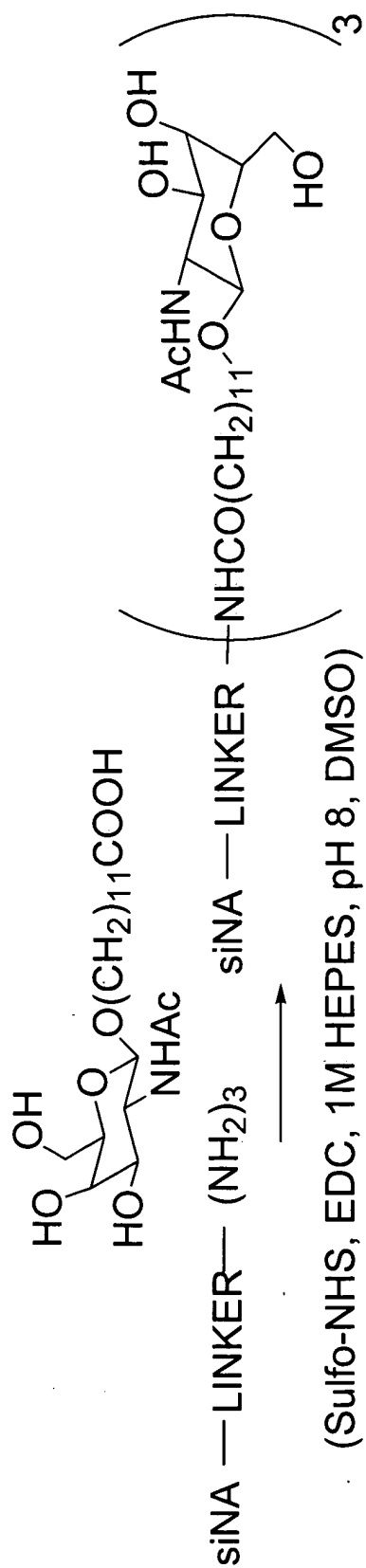
siNA= short interfering nucleic acid molecule or a portion thereof

**Figure 52: siNA Phospholipid Conjugate**

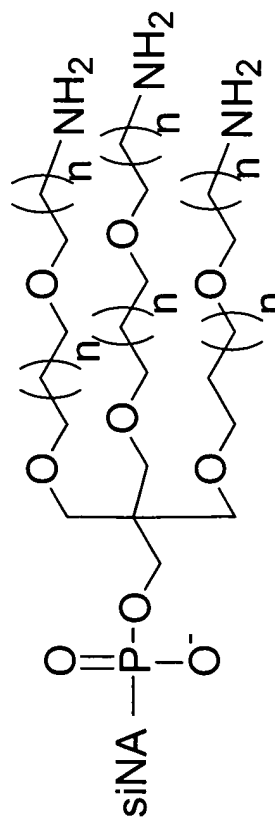


CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT

**Figure 53: siNA-NAcGalactosamine post-synthetic coupling**

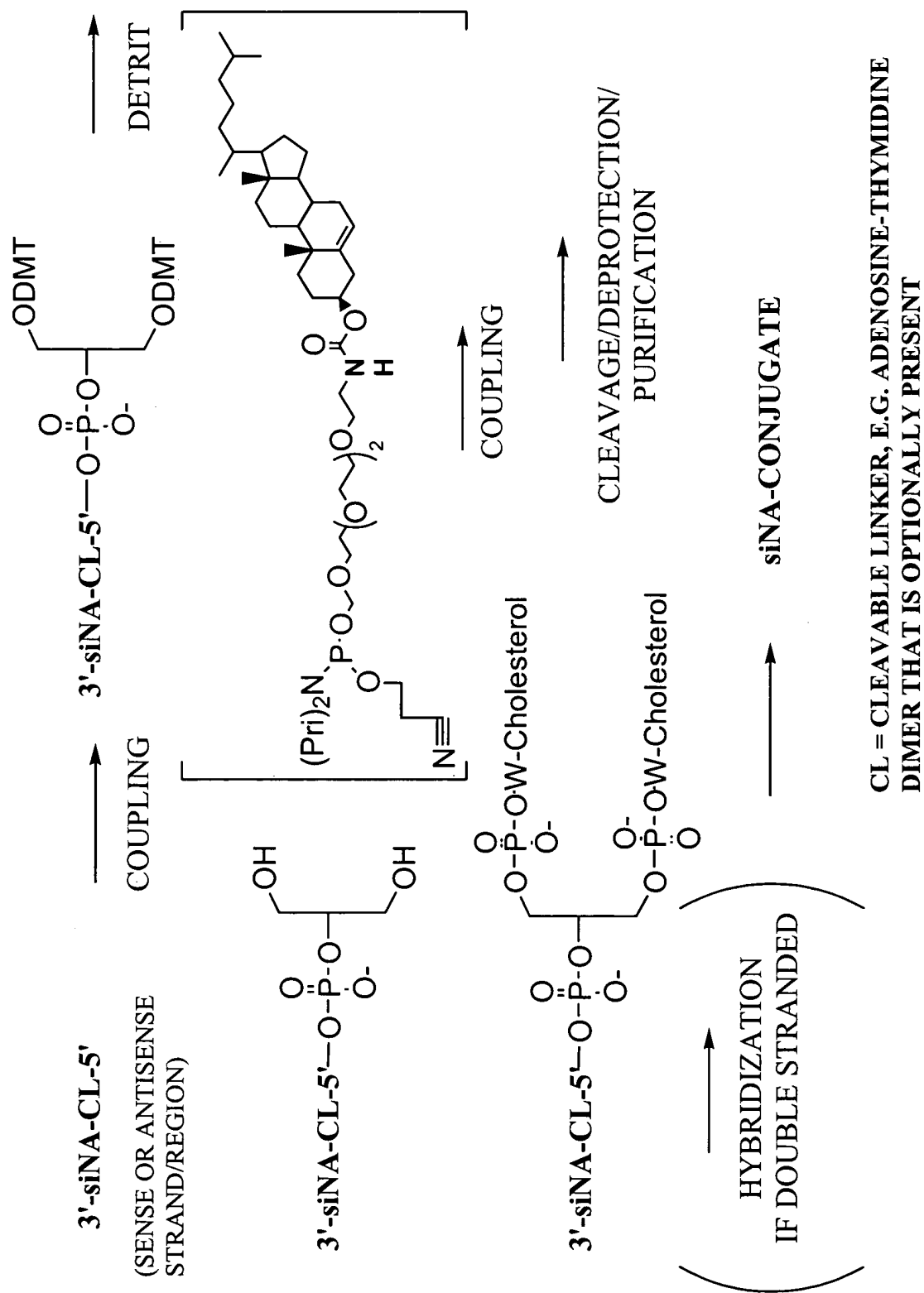


FOR EXAMPLE: OLIGO-LINKER =



Where n is an integer from 1 to 20

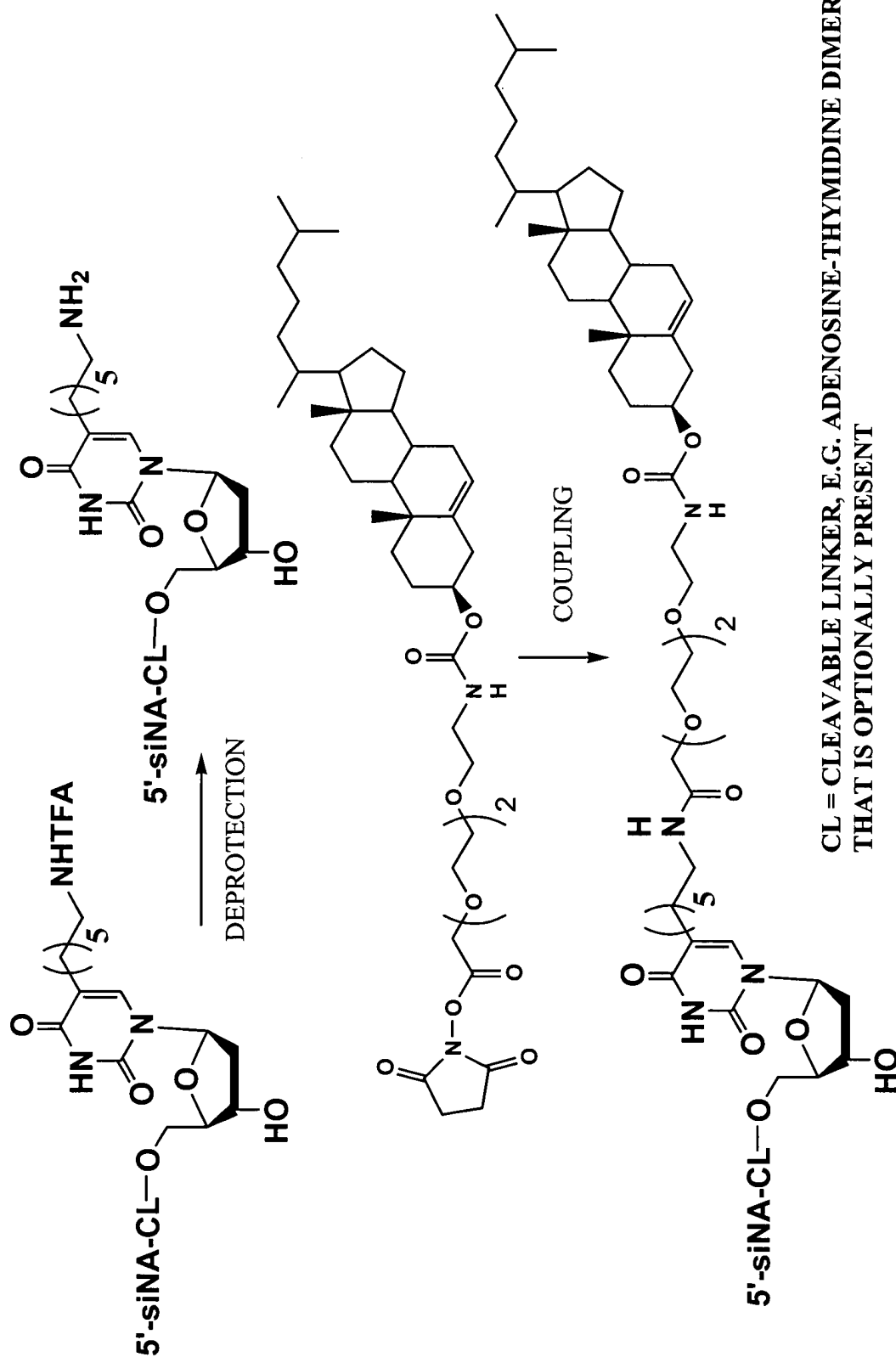
**Figure 54: siNA Cholesterol Conjugate**



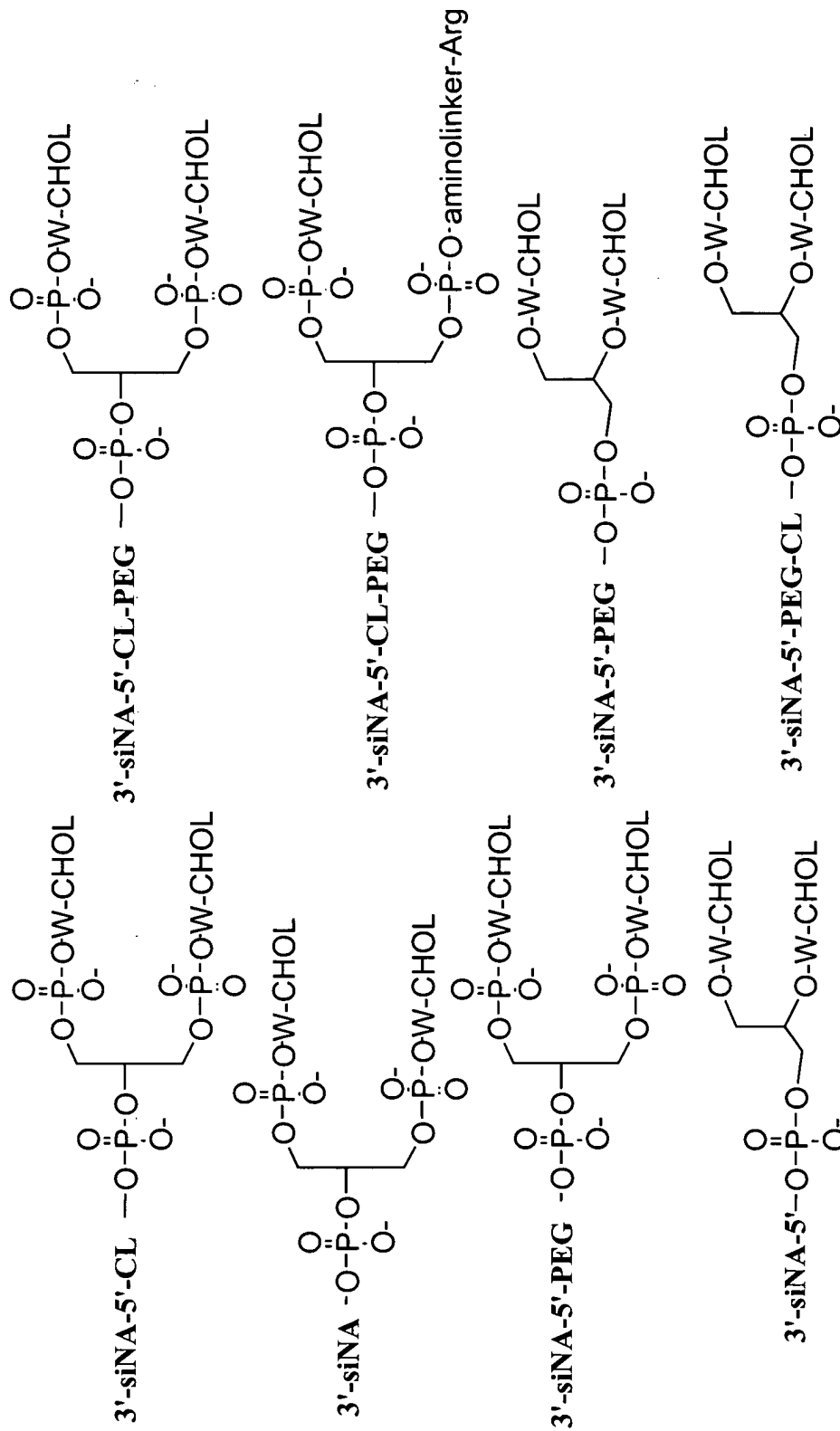
Chemical reaction scheme showing the deprotection of a 5'-siRNA-CL-O- derivative. The starting material is a 5'-siRNA-CL-O- derivative with a 5'-(NHTFA) group. An arrow labeled "DEPROTECTION" points to the product, which is a 5'-siRNA-CL-O- derivative with a 5'-(K-PEG) group. The product is shown in brackets, indicating it is a mixture of isomers.

**CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT**

**Figure 56: siNA 3'-Cholesterol Conjugate**



**Figure 57: Nucleic Acid Cholesterol Conjugates**



PEG=polyethylene glycol

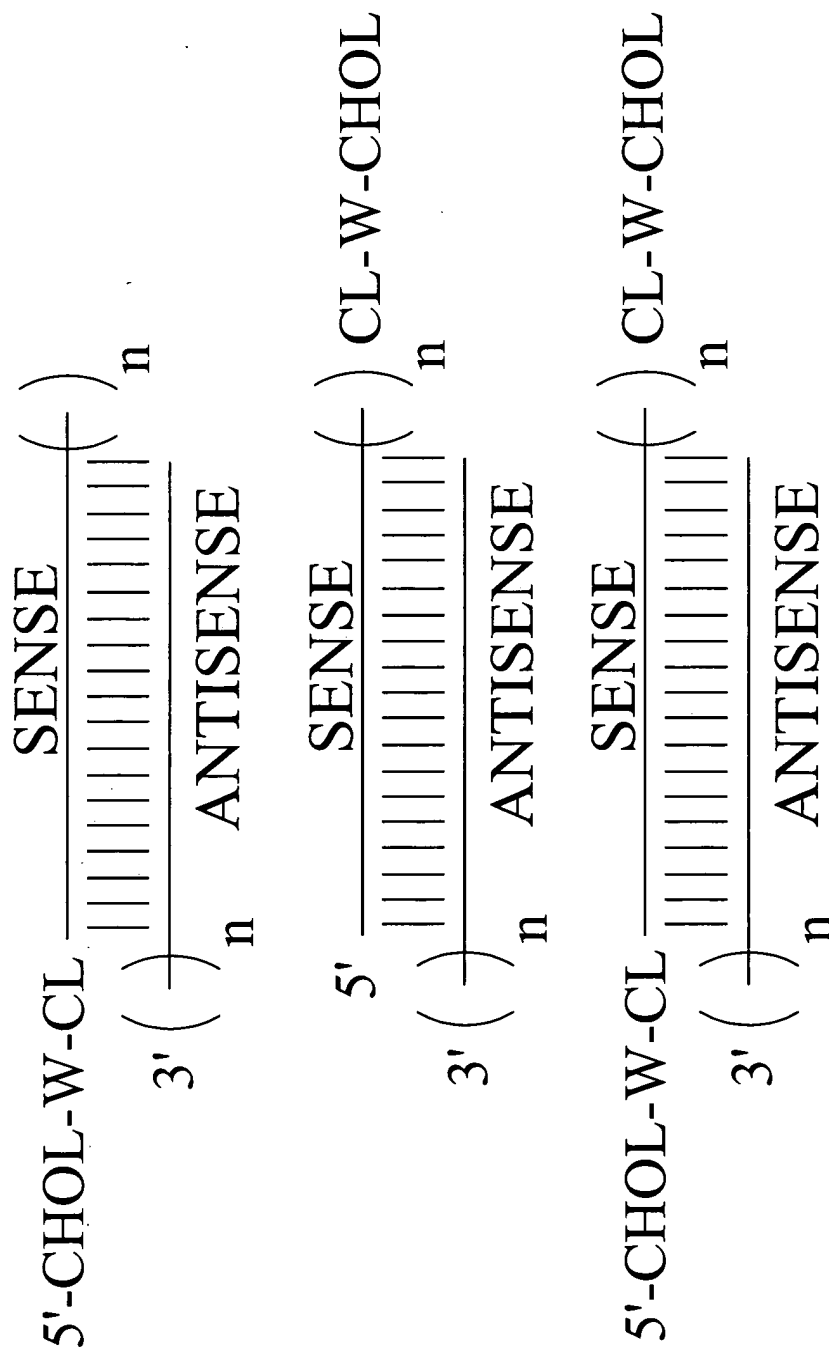
CL=cleavable linker (e.g. A-dT, C-dT)

siNA= short interfering nucleic acid molecule or a portion thereof

CHOL=cholesterol or an analog or metabolite thereof

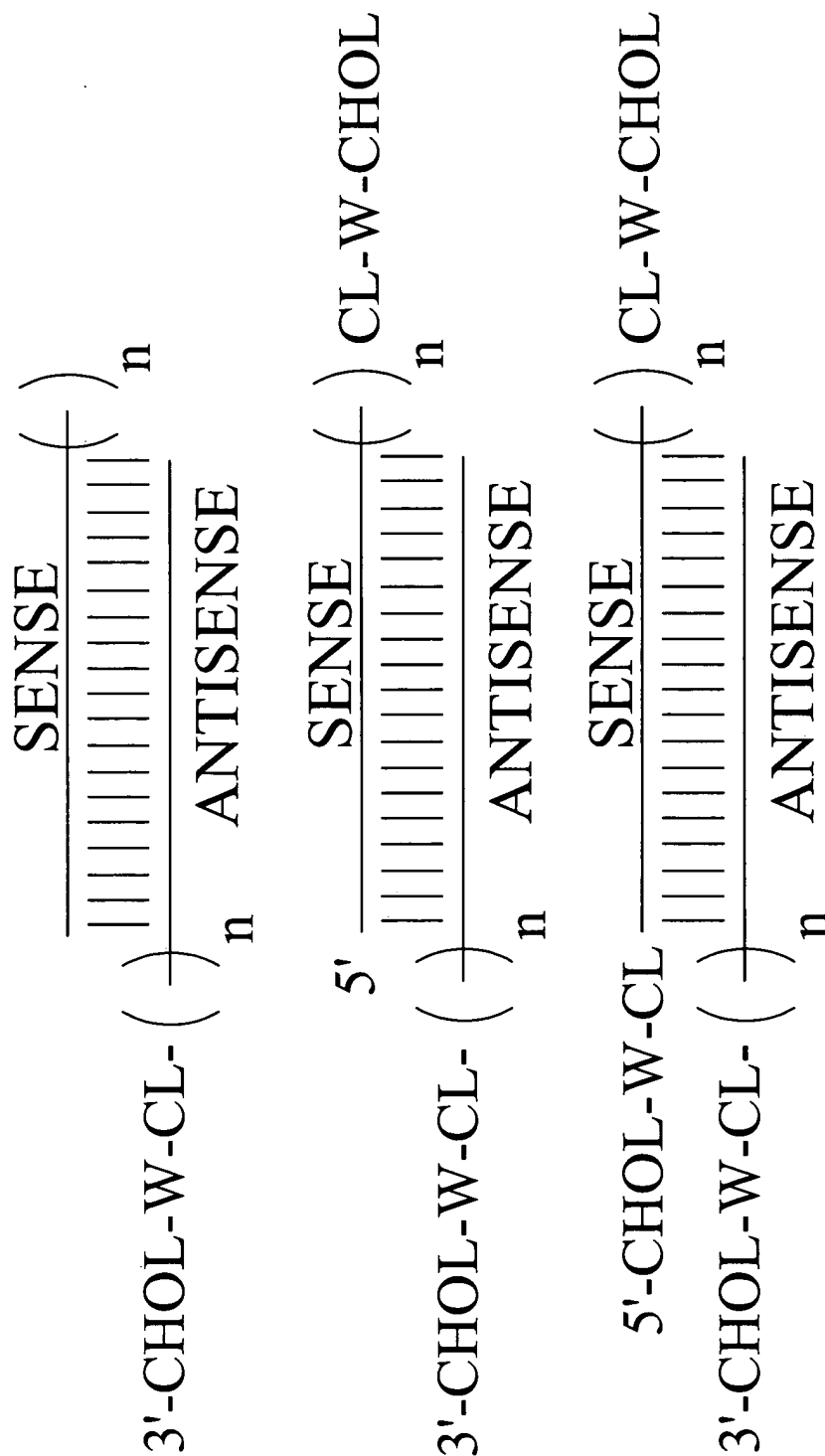
W= linker molecule (see for example Formulae 109 or 112)

**Figure 58: siNA Cholesterol Conjugates**



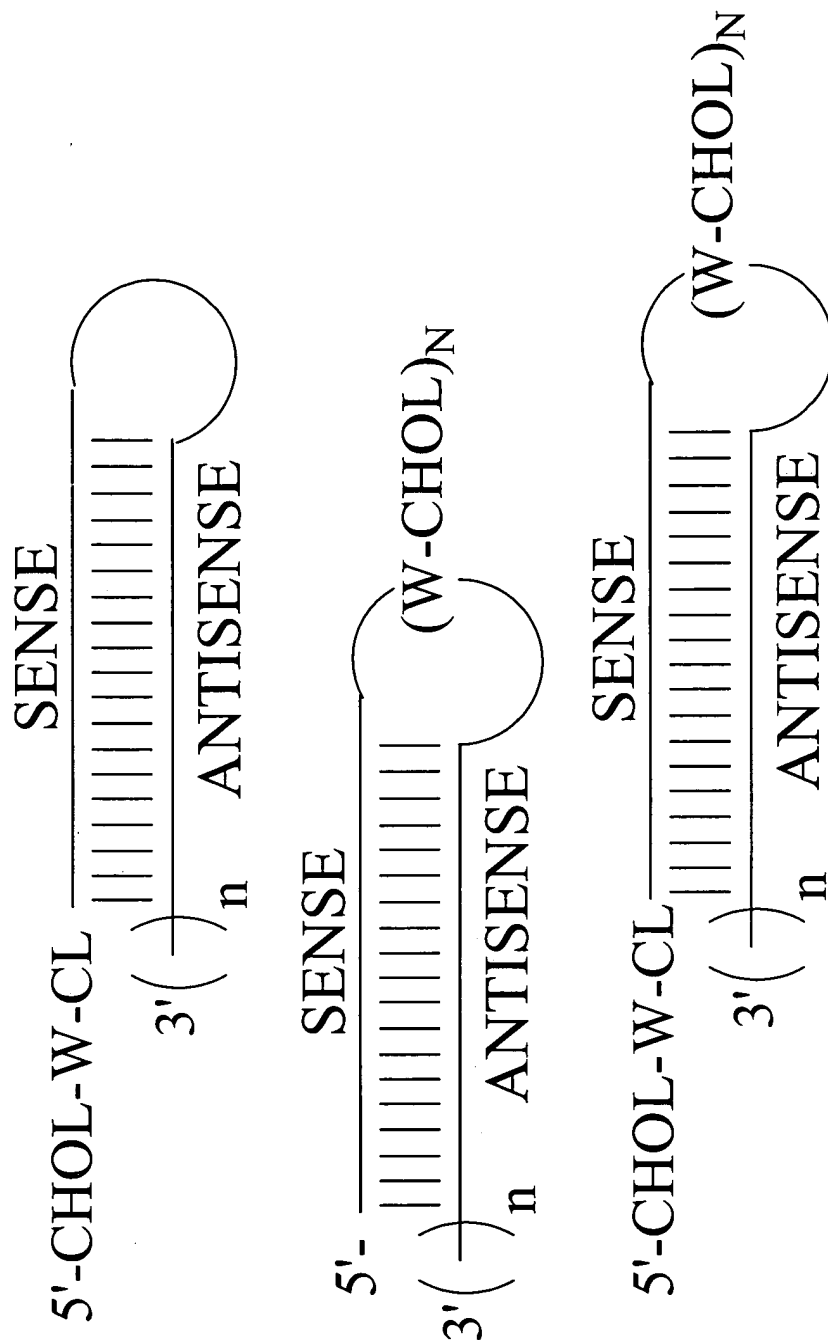
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present  
 CHOL=cholesterol or an analog or metabolite thereof  
 W= linker molecule (see for example Formulae 107, 108, 109 or 115)  
 n = integer, e.g. 1, 2, or 3

**Figure 59: siNA Cholesterol Conjugates**



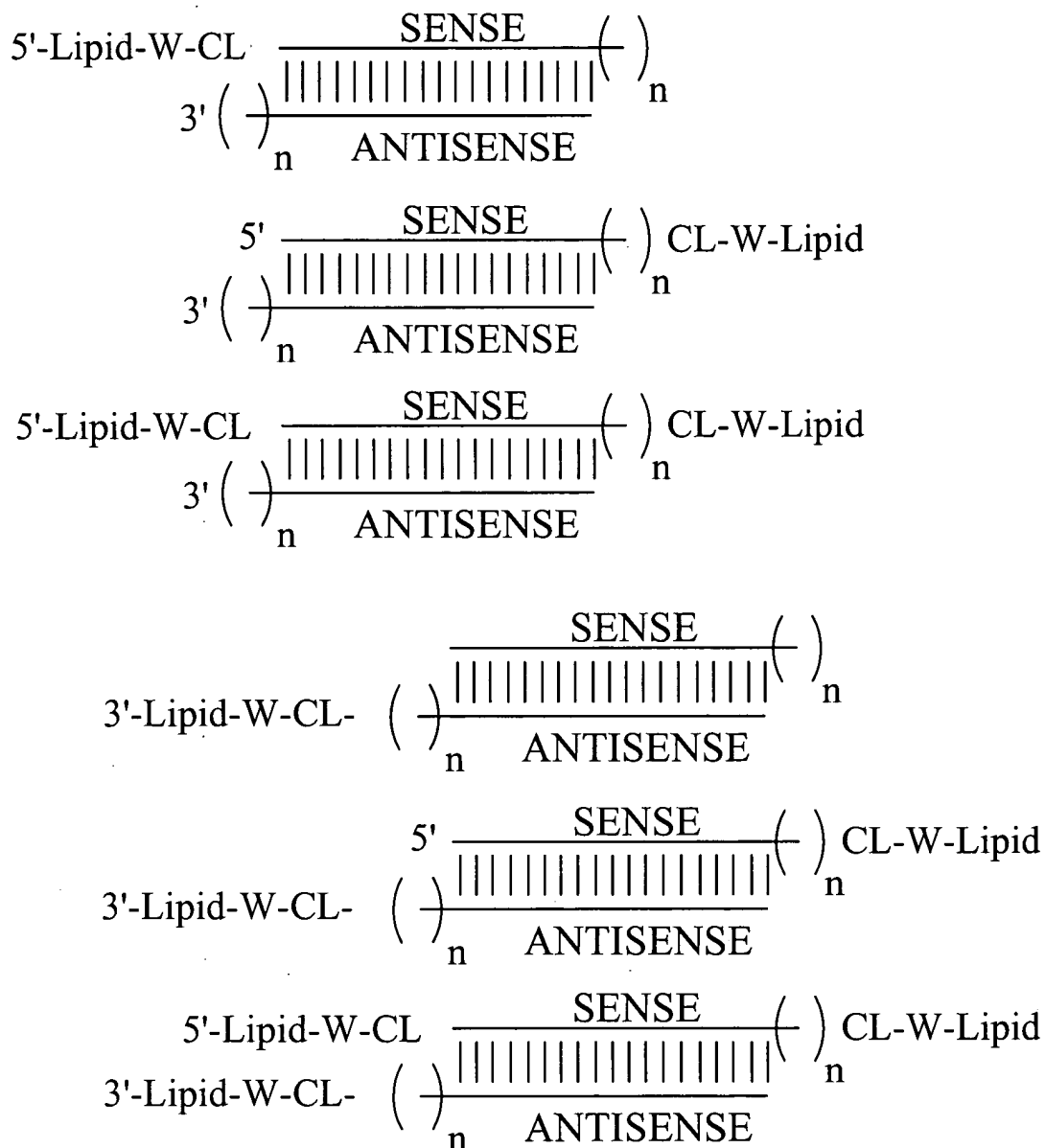
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present  
 CHOL=cholesterol or an analog or metabolite thereof  
 W= linker molecule (see for example Formulae 107, 108, 109 or 115)  
 n = integer, e.g. 1, 2, or 3

**Figure 60: siNA Cholesterol Conjugates**



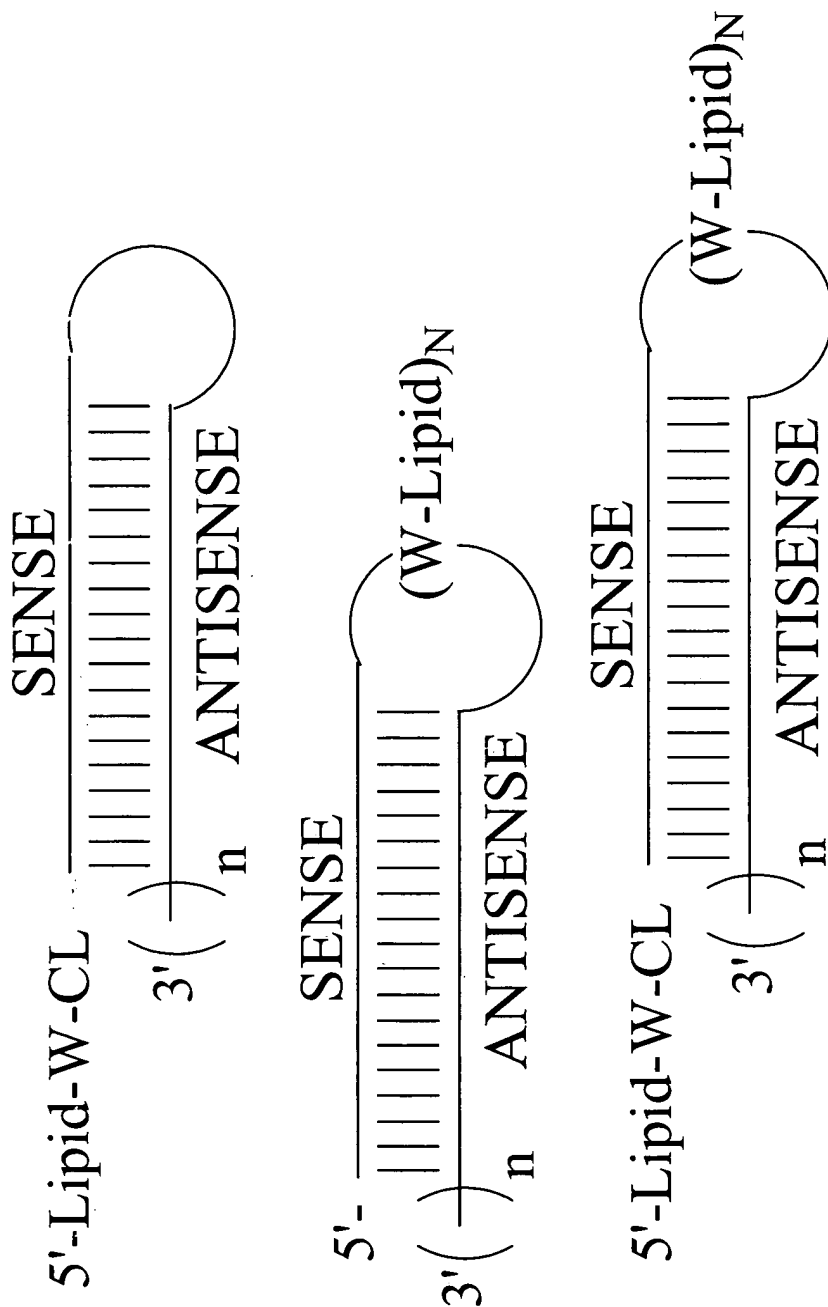
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present  
 CHOL=cholesterol or an analog or metabolite thereof  
 W= linker molecule (see for example Formulae 107, 108, 109 or 112)  
 $n$  = integer, e.g. 1, 2, or 3  
 $N$ =integer, e.g. 1, 2, 3, or 4

## Figure 61: siNA Lipid Conjugates



**CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present**  
**Lipid=Straight chain or branched alkyl or fatty acid, e.g. C<sub>18</sub>H<sub>37</sub>**  
**W= linker molecule (see for example Formulae 48, 49, 64, or 65)**  
**n = integer, e.g. 1, 2, or 3**

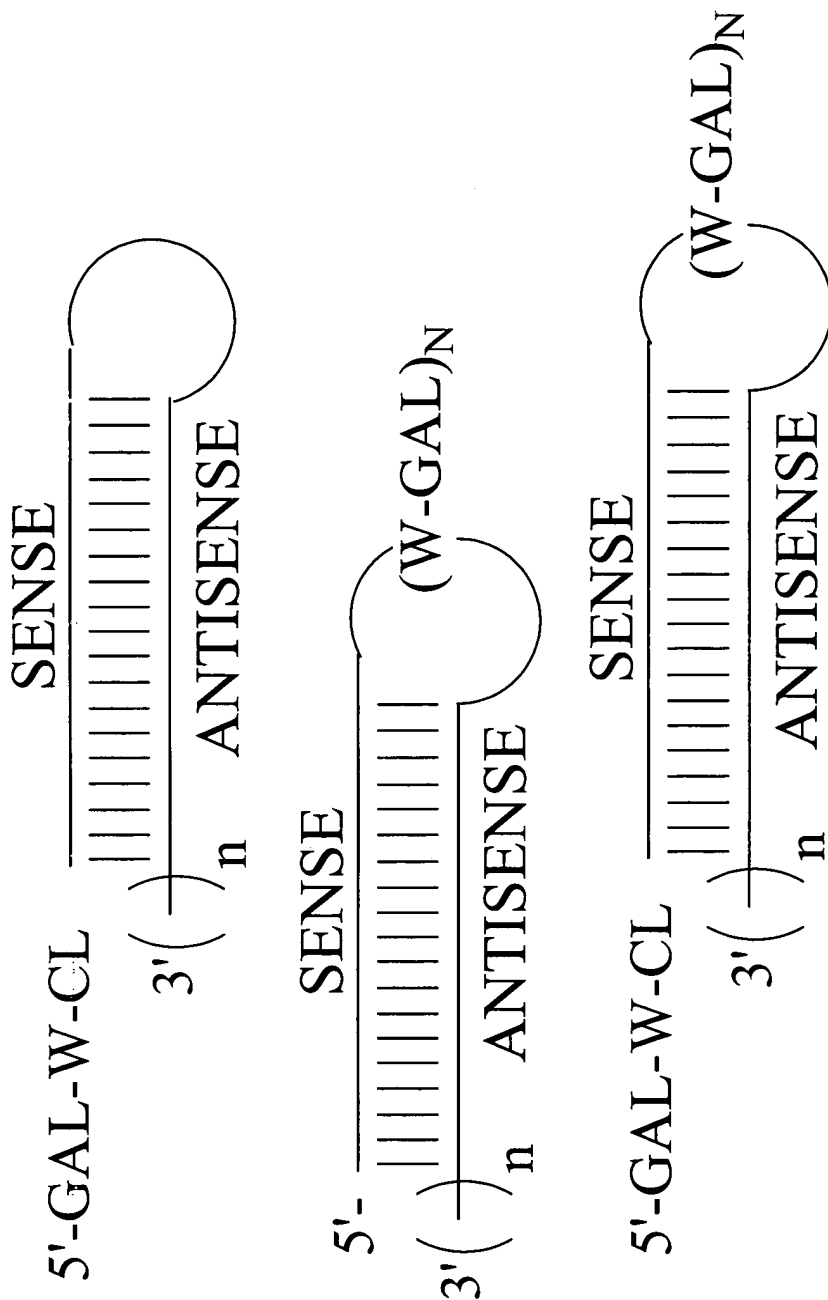
**Figure 62: siNA Lipid Conjugates**



CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present  
 Lipid=Straight chain or branched alkyl or fatty acid, e.g. C<sub>18</sub>H<sub>37</sub>  
 W= linker molecule (see for example Formulae 48, 49, 64, or 65)  
 n = integer, e.g. 1, 2, or 3  
 N=integer, e.g. 1, 2, 3, or 4

**n = integer, e.g. 1, 2, or 3**

**Figure 64: siNA Galactosamine Conjugates**



CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present  
 GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106  
 W= linker molecule (see for example Formulae 102 or 103)  
 n = integer, e.g. 1, 2, or 3  
 N=integer, e.g. 1, 2, 3, or 4

5'-CONJ-W-CL  $\frac{\text{SENSE}}{\left( \frac{\text{|||||}}{\text{ANTISENSE}} \right)_n}$

3'  $\left( \frac{\text{|||||}}{\text{ANTISENSE}} \right)_n$  CL-W-CONJ

5'-CONJ-W-CL  $\frac{\text{SENSE}}{\left( \frac{\text{|||||}}{\text{ANTISENSE}} \right)_n}$  CL-W-CONJ

3'  $\left( \frac{\text{|||||}}{\text{ANTISENSE}} \right)_n$  CL-W-CONJ

3'-CONJ-W-CL-  $\left( \frac{\text{SENSE}}{\left( \frac{\text{|||||}}{\text{ANTISENSE}} \right)_n} \right)_n$

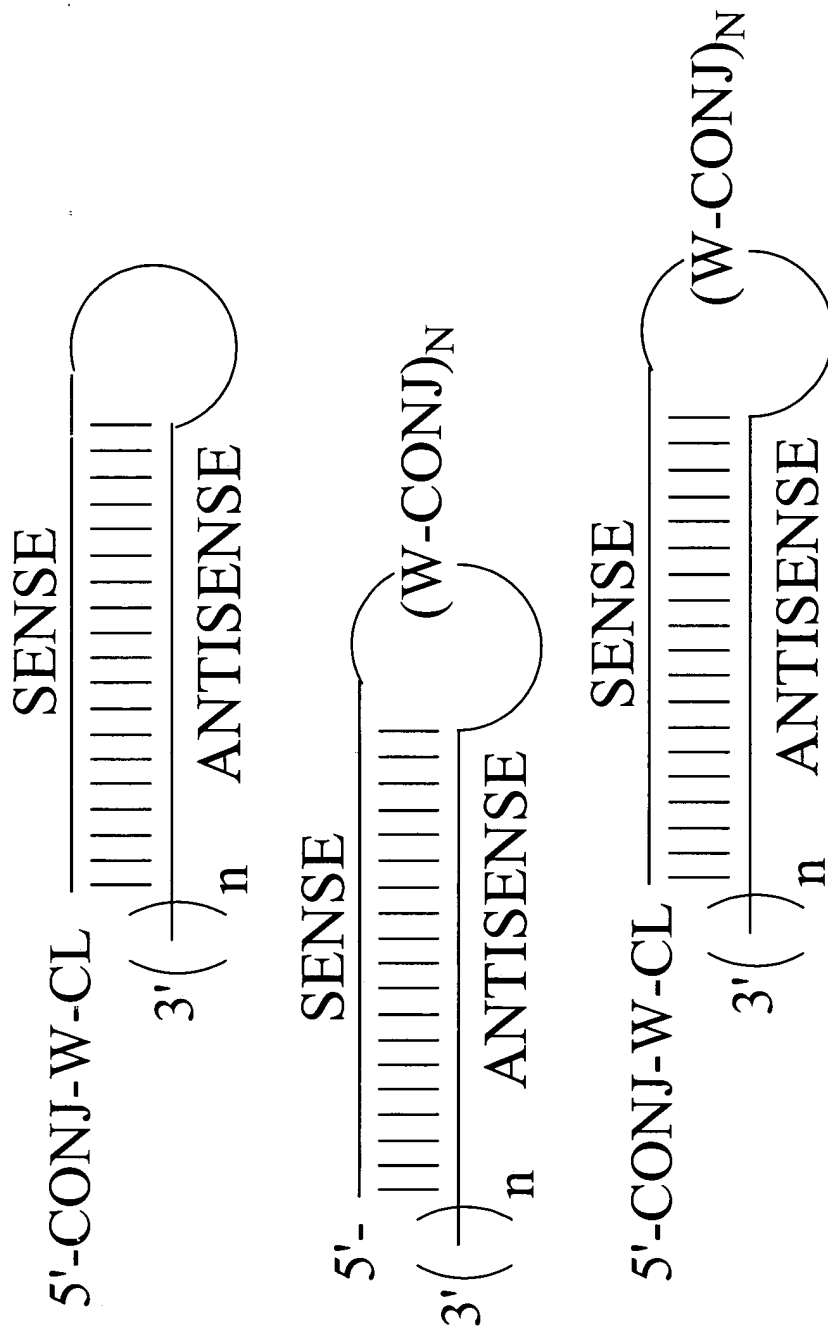
3'-CONJ-W-CL-  $\left( \frac{\text{SENSE}}{\left( \frac{\text{|||||}}{\text{ANTISENSE}} \right)_n} \right)_n$  CL-W-CONJ

5'-CONJ-W-CL  $\frac{\text{SENSE}}{\left( \frac{\text{|||||}}{\text{ANTISENSE}} \right)_n}$  CL-W-CONJ

3'-CONJ-W-CL-  $\left( \frac{\text{|||||}}{\text{ANTISENSE}} \right)_n$

**n = integer, e.g. 1, 2, or 3**

**Figure 66: Generalized siNA Conjugate design**



CONJ=any biologically active molecule or conjugate as described herein

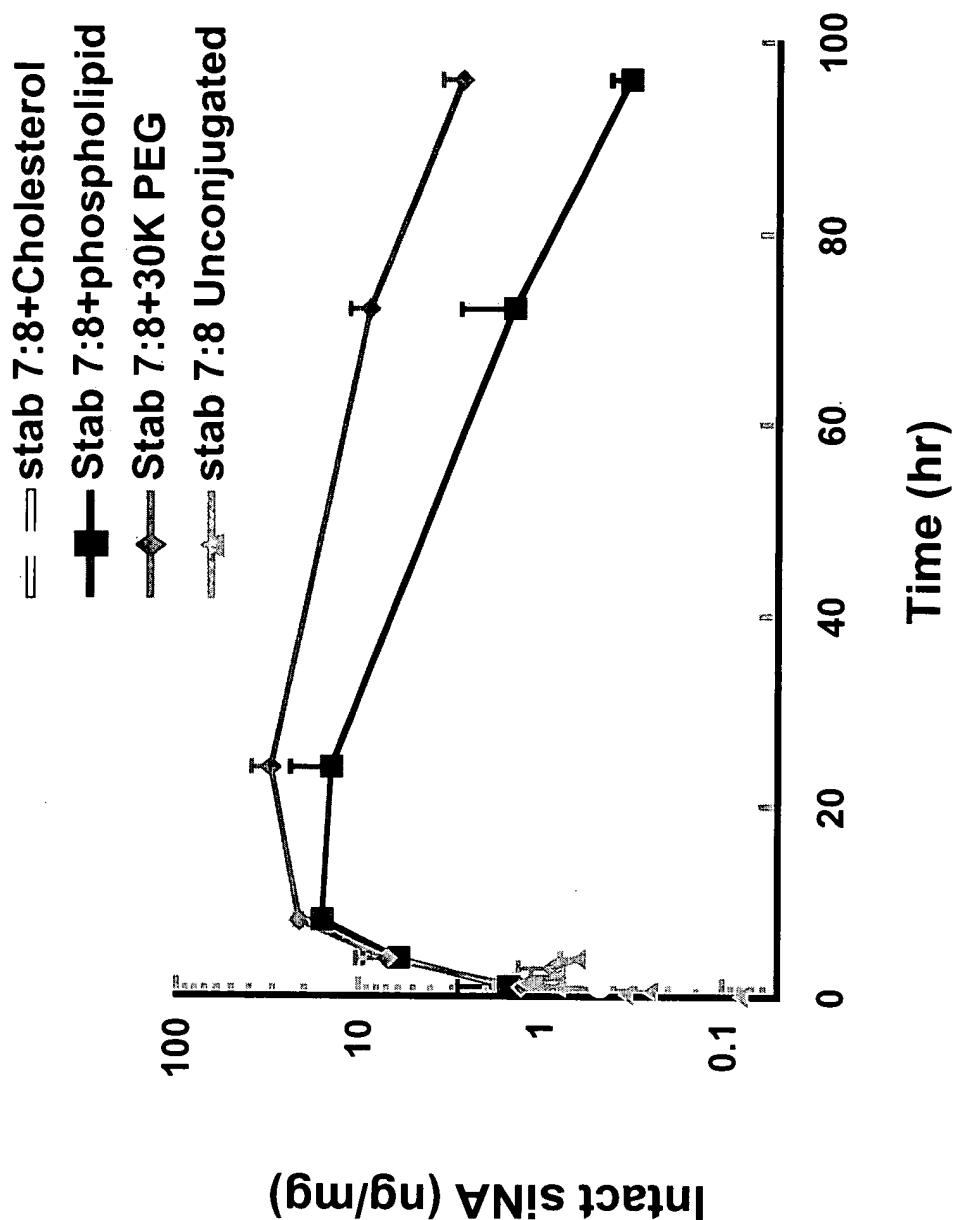
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present

W= linker molecule

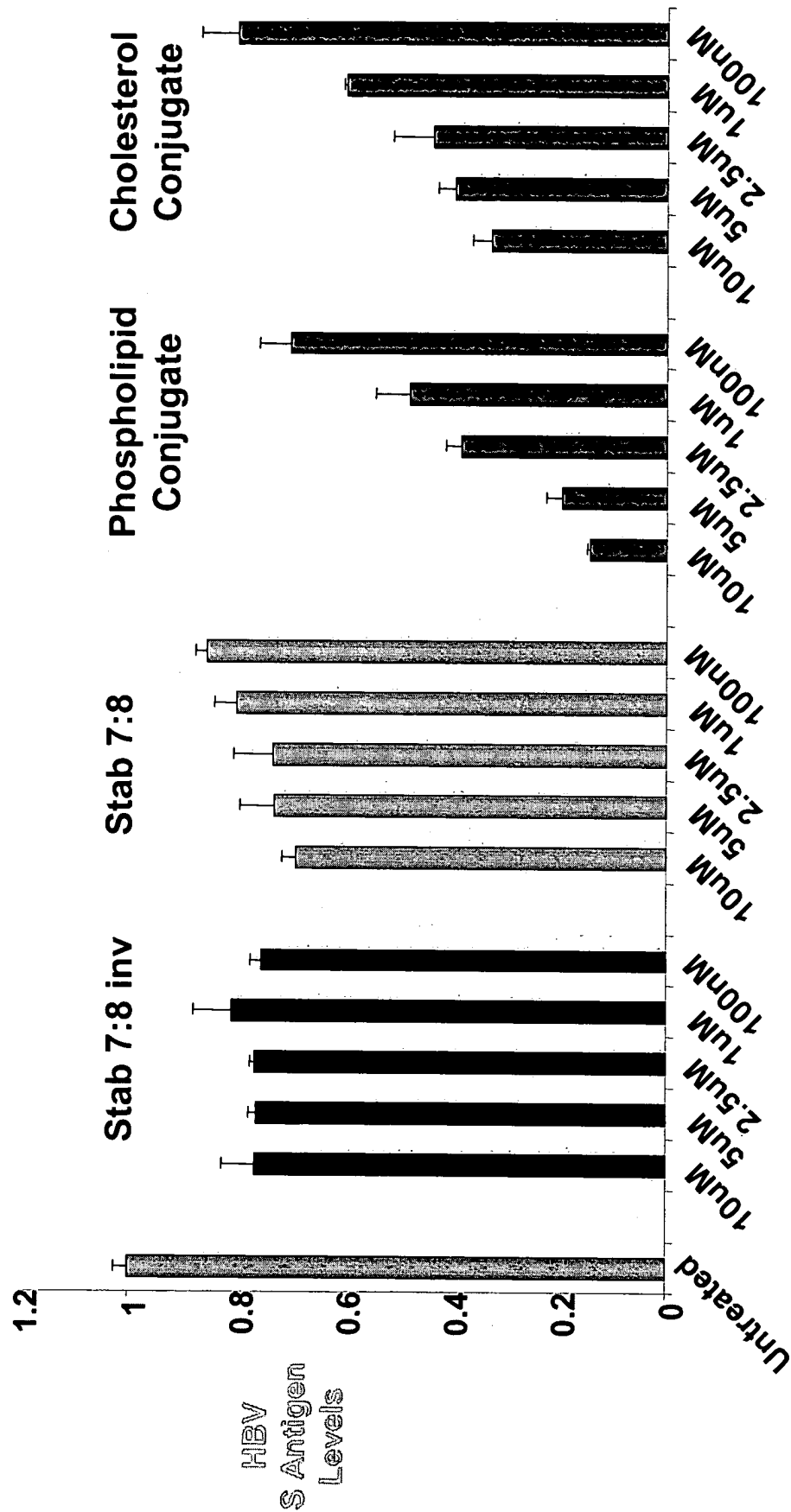
n = integer, e.g. 1, 2, or 3

N=integer, e.g. 1, 2, 3, or 4

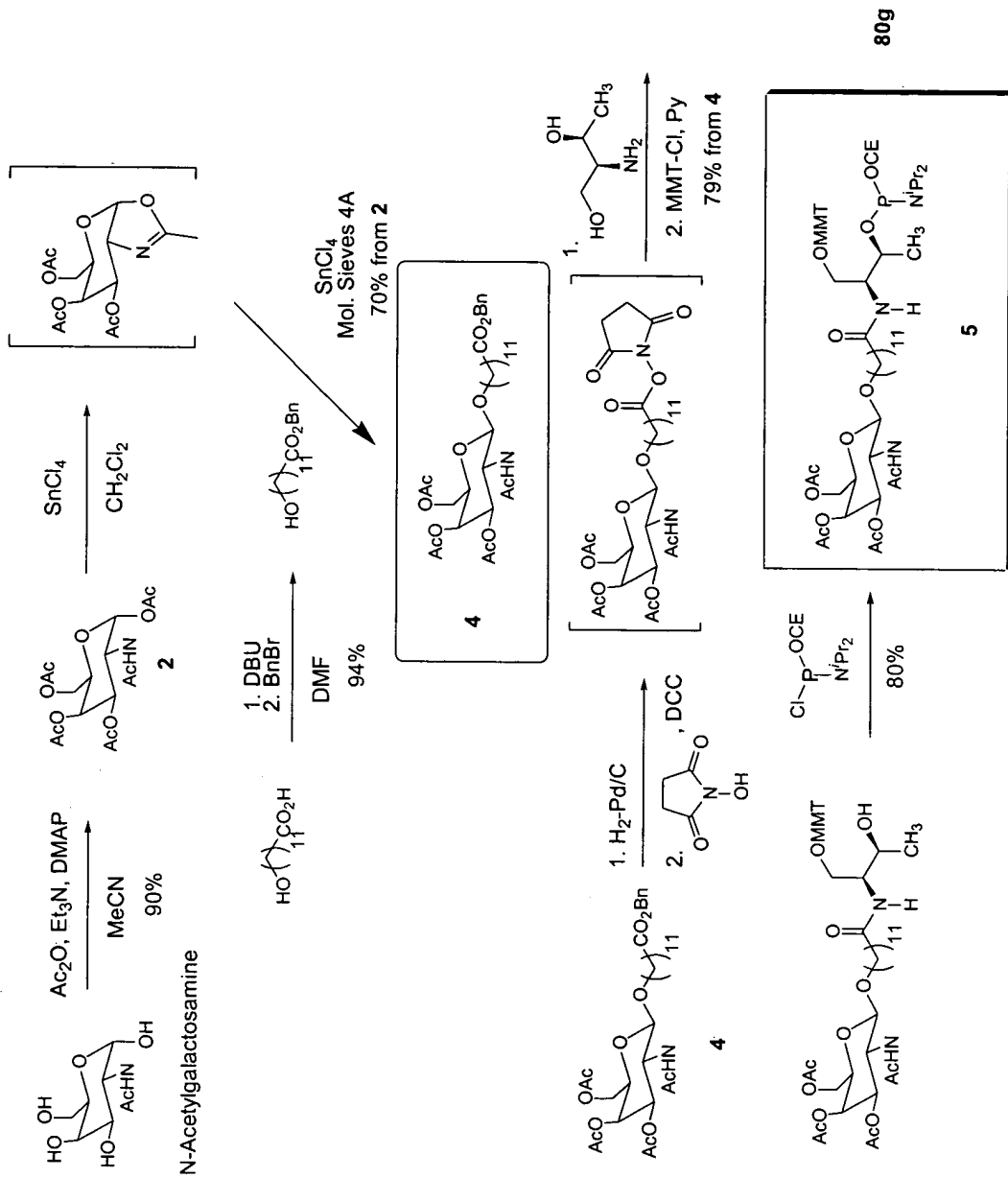
**Figure 67: Distribution of Intact siNA in Liver After SC Administration of Conjugated or Unconjugated Chemistries**



**Figure 68: Lipid Free Delivery of HBV siNA Conjugates in Cell Culture**

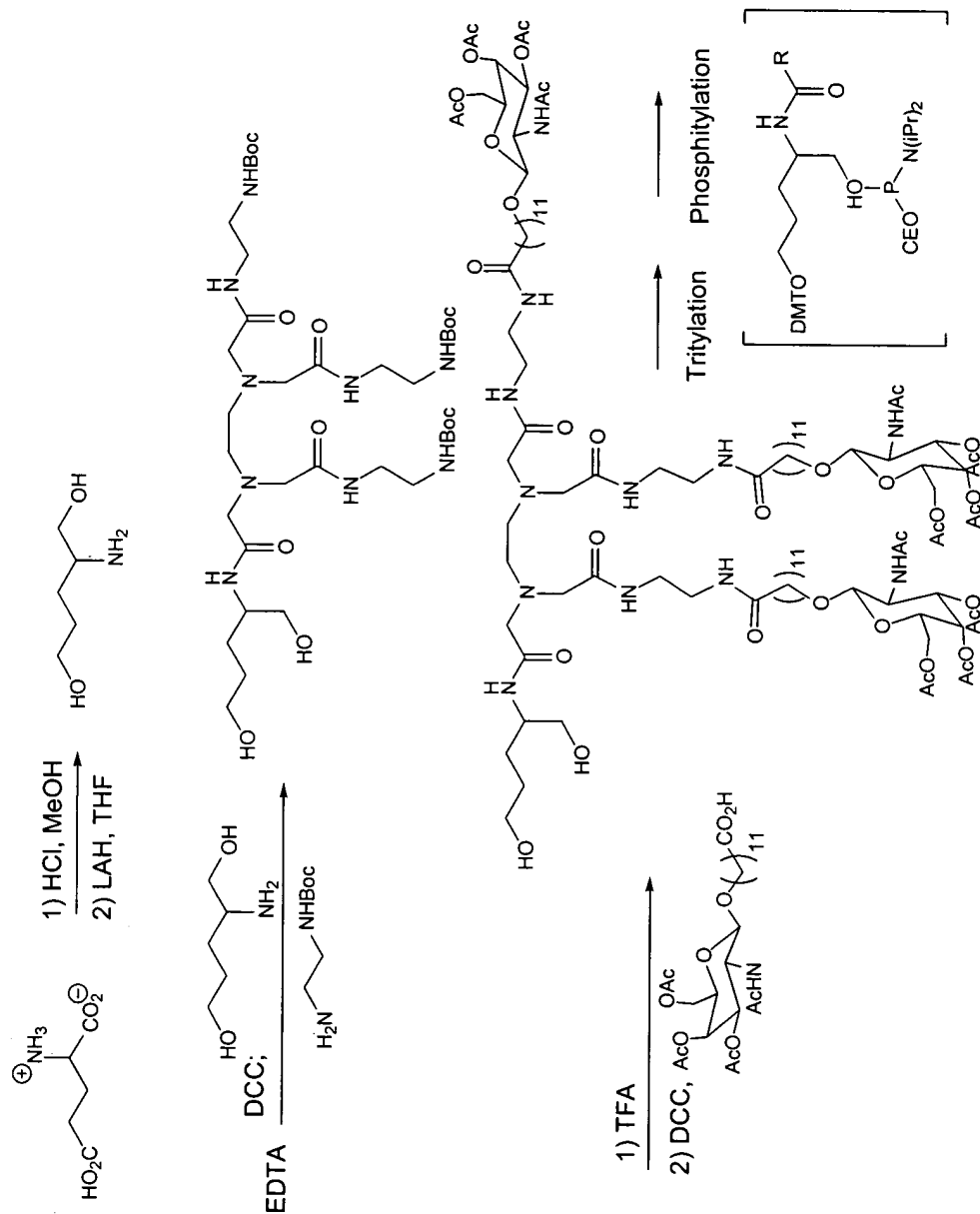


**Figure 69: Scale-up of “mono” Galactosamine phosphoramidite**

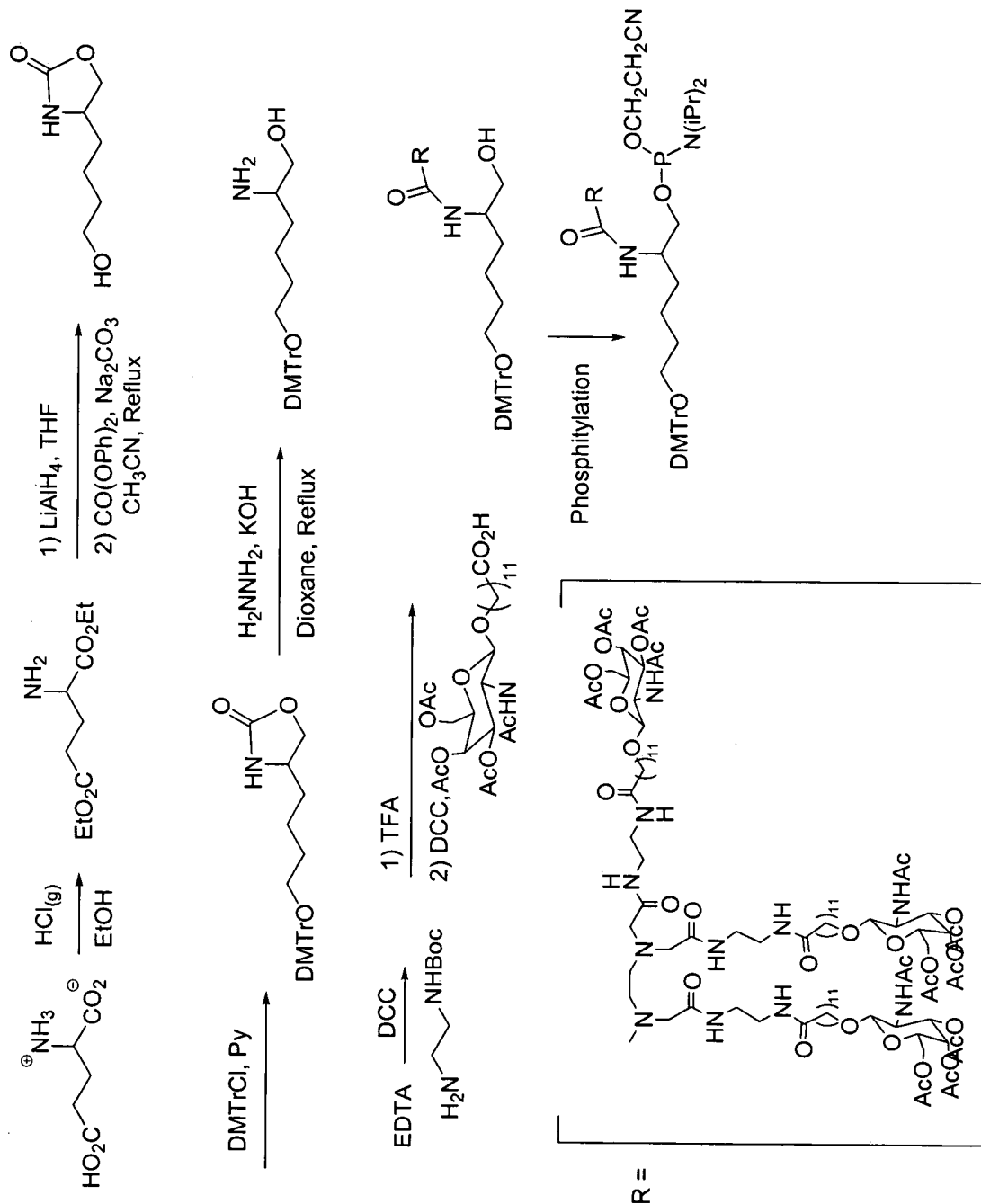


[illegible]

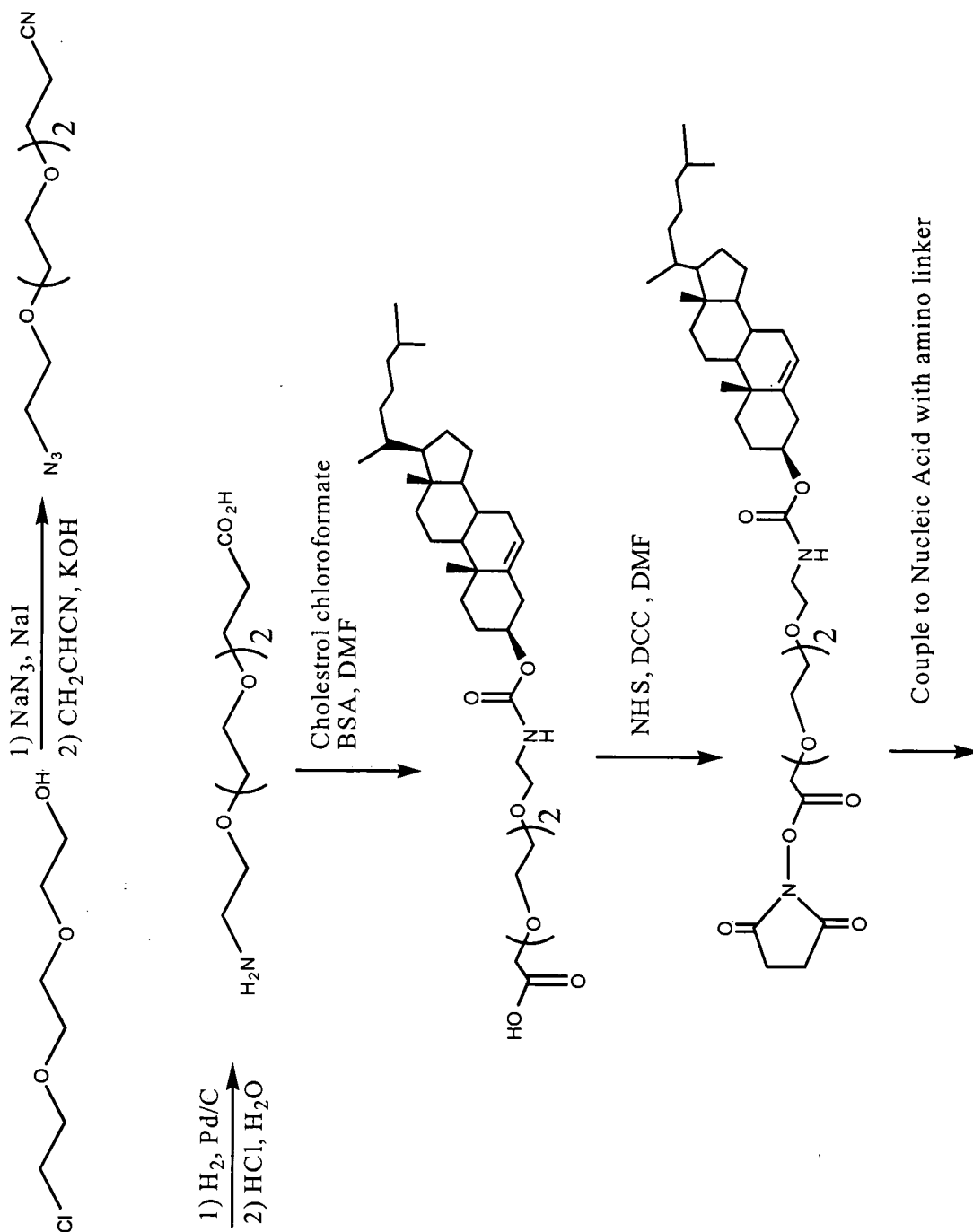
**Figure 71: Synthesis of another Tri-Galactosamine Conjugate**



**Figure 72: Alternate Synthesis of Tri-Galactosamine Conjugate**



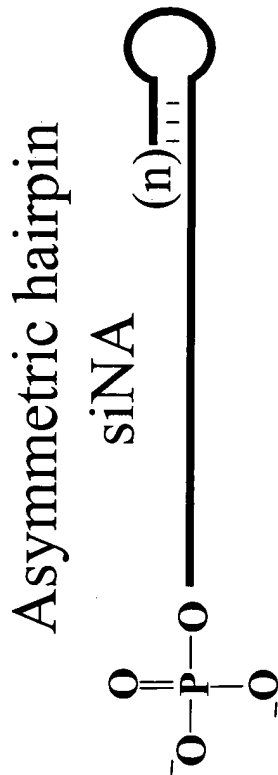
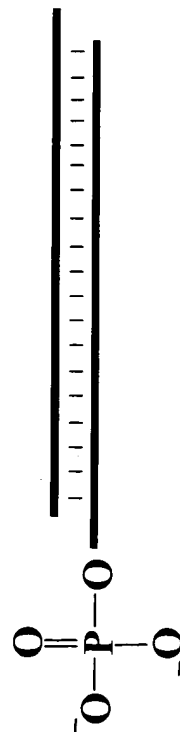
**Figure 73: Synthesis of NHS Cholesterol Conjugate**



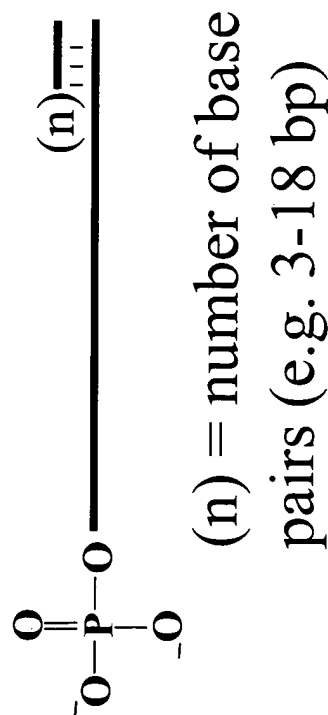
**Figure 74: Phosphorylated siNA constructs**



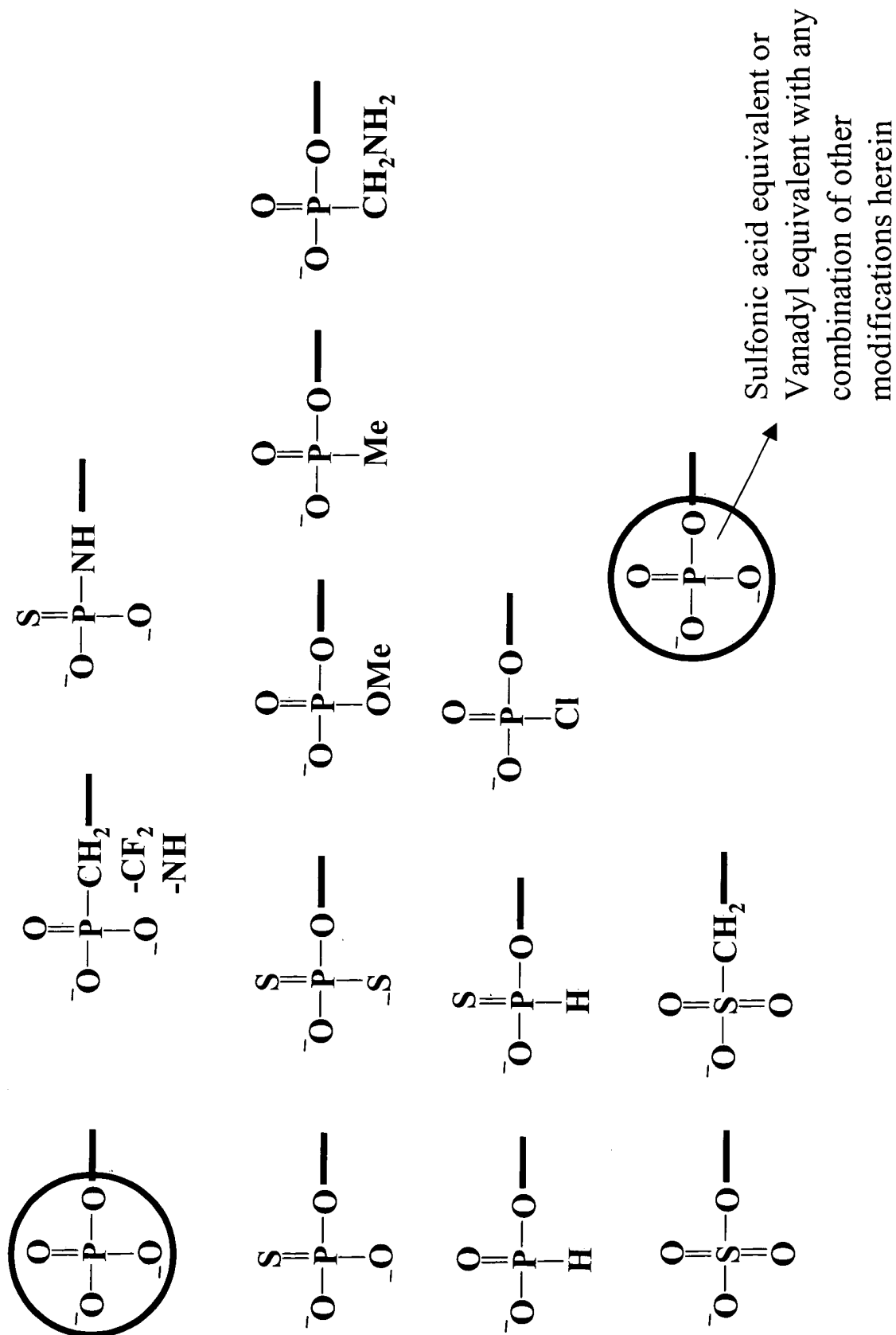
Phosphates can be modified  
as described herein



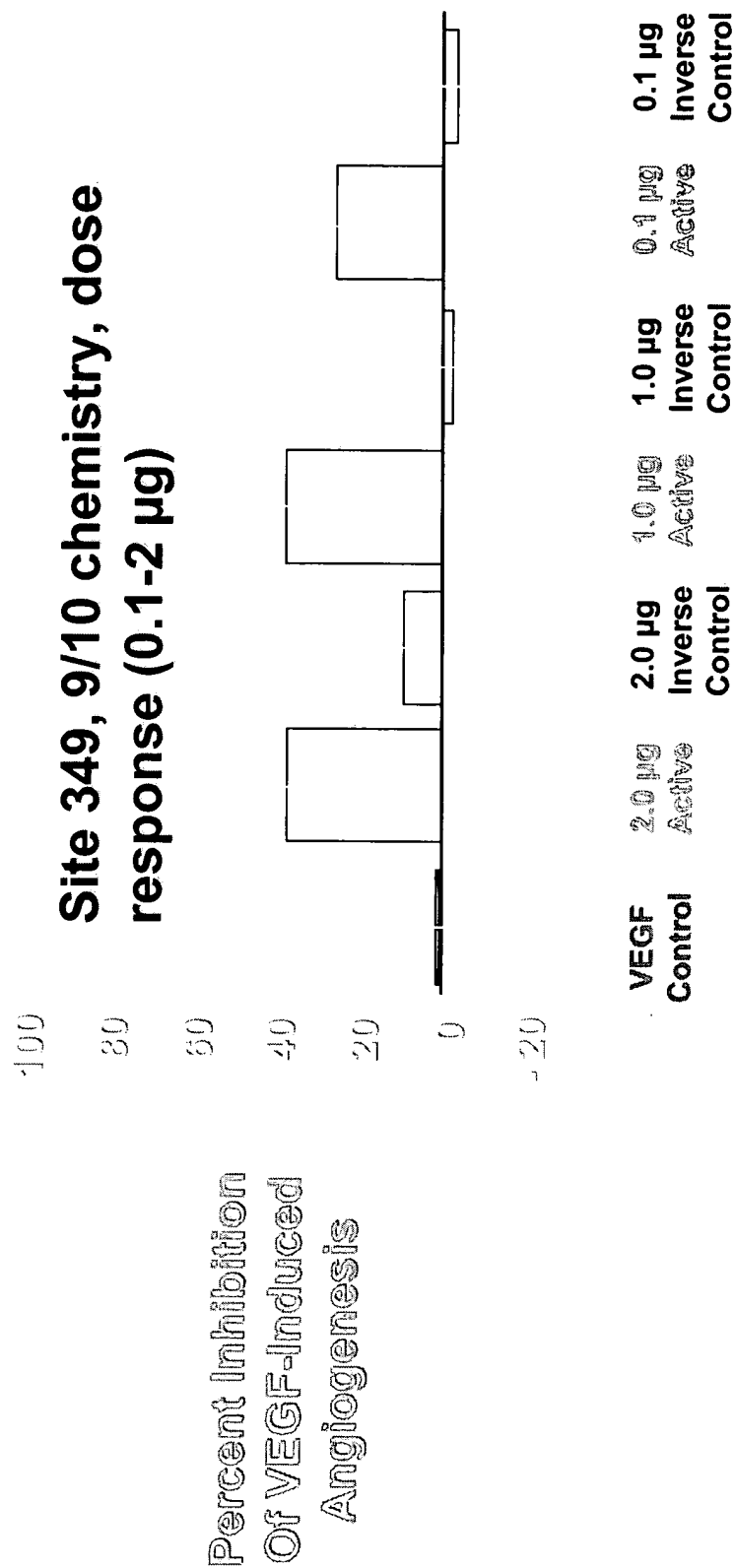
Asymmetric duplex  
siNA



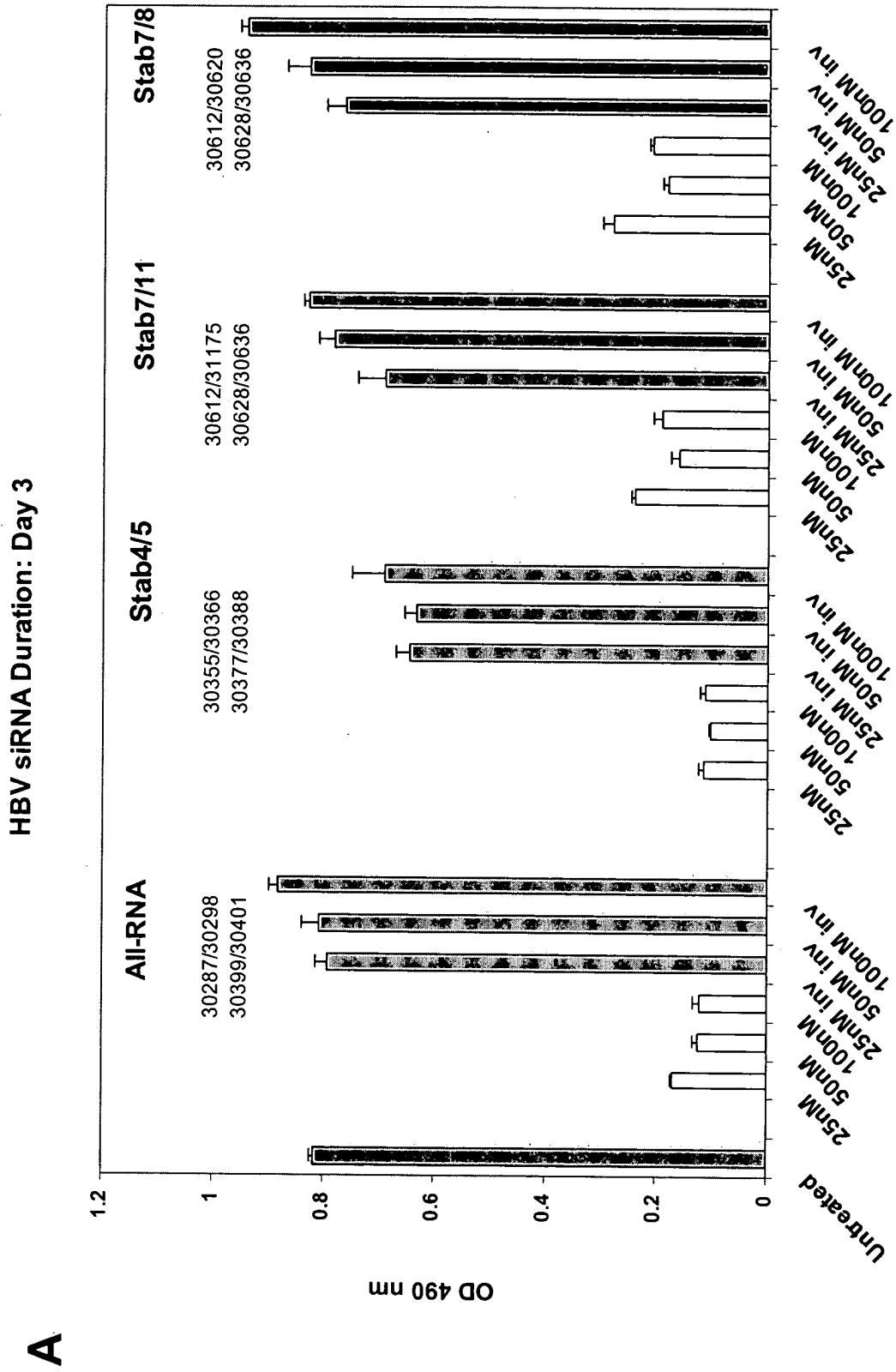
**Figure 75: 5'-phosphate modifications**



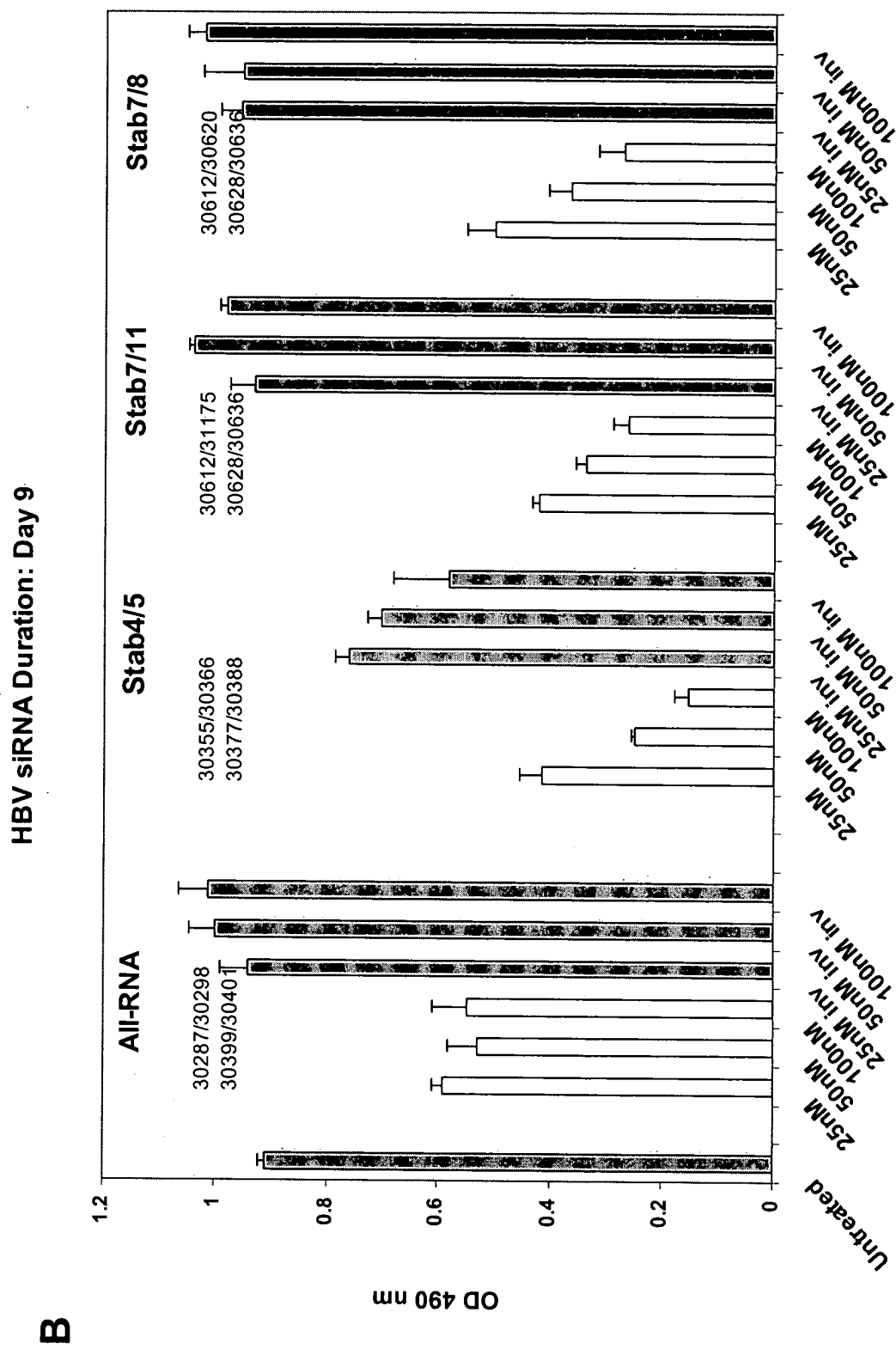
**Figure 76: siNA Targeting VEGFR-1 Inhibits VEGF-Induced Rat Corneal Angiogenesis**



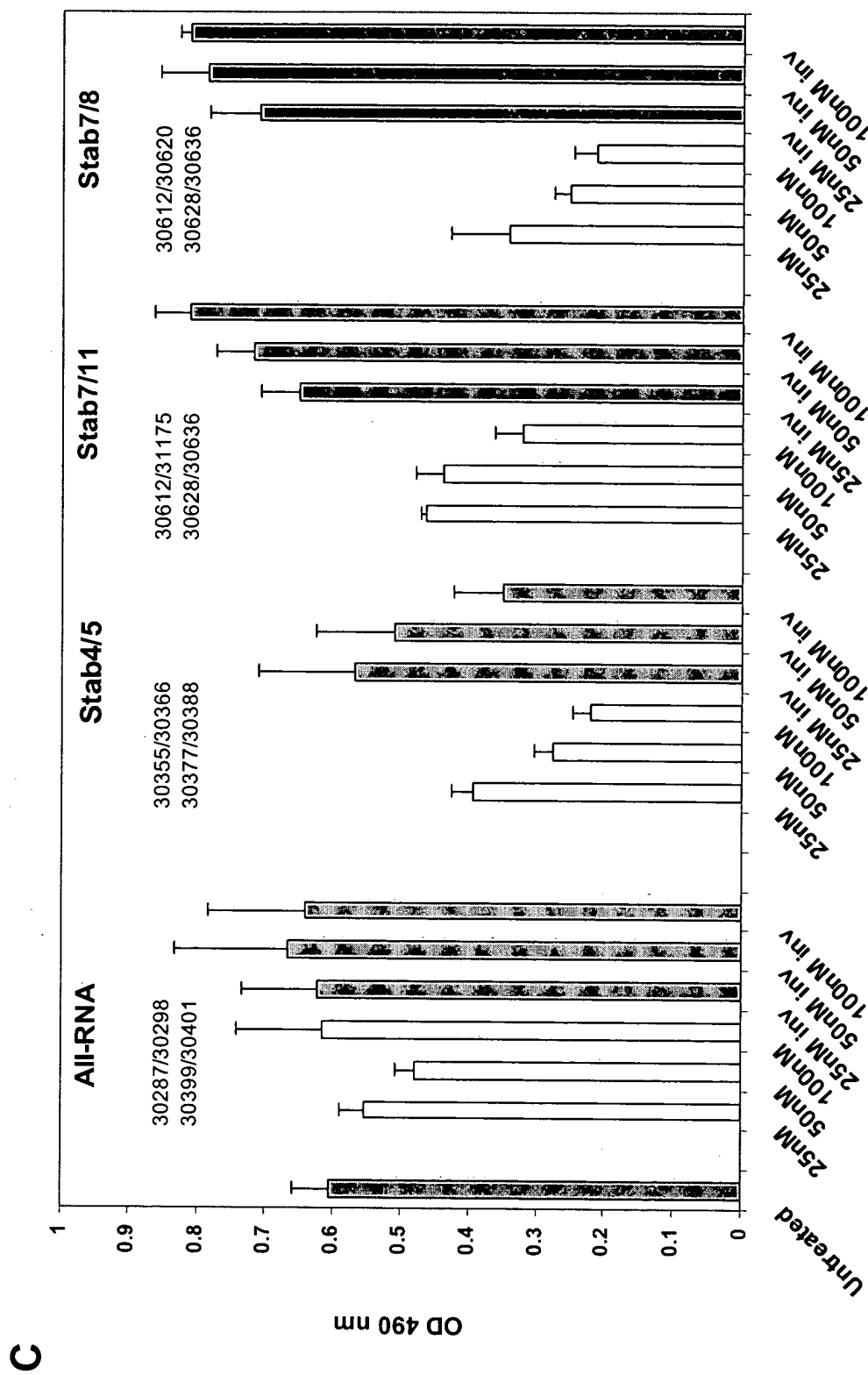
**Figure 77: Duration of Effect of Modified siNA Constructs**



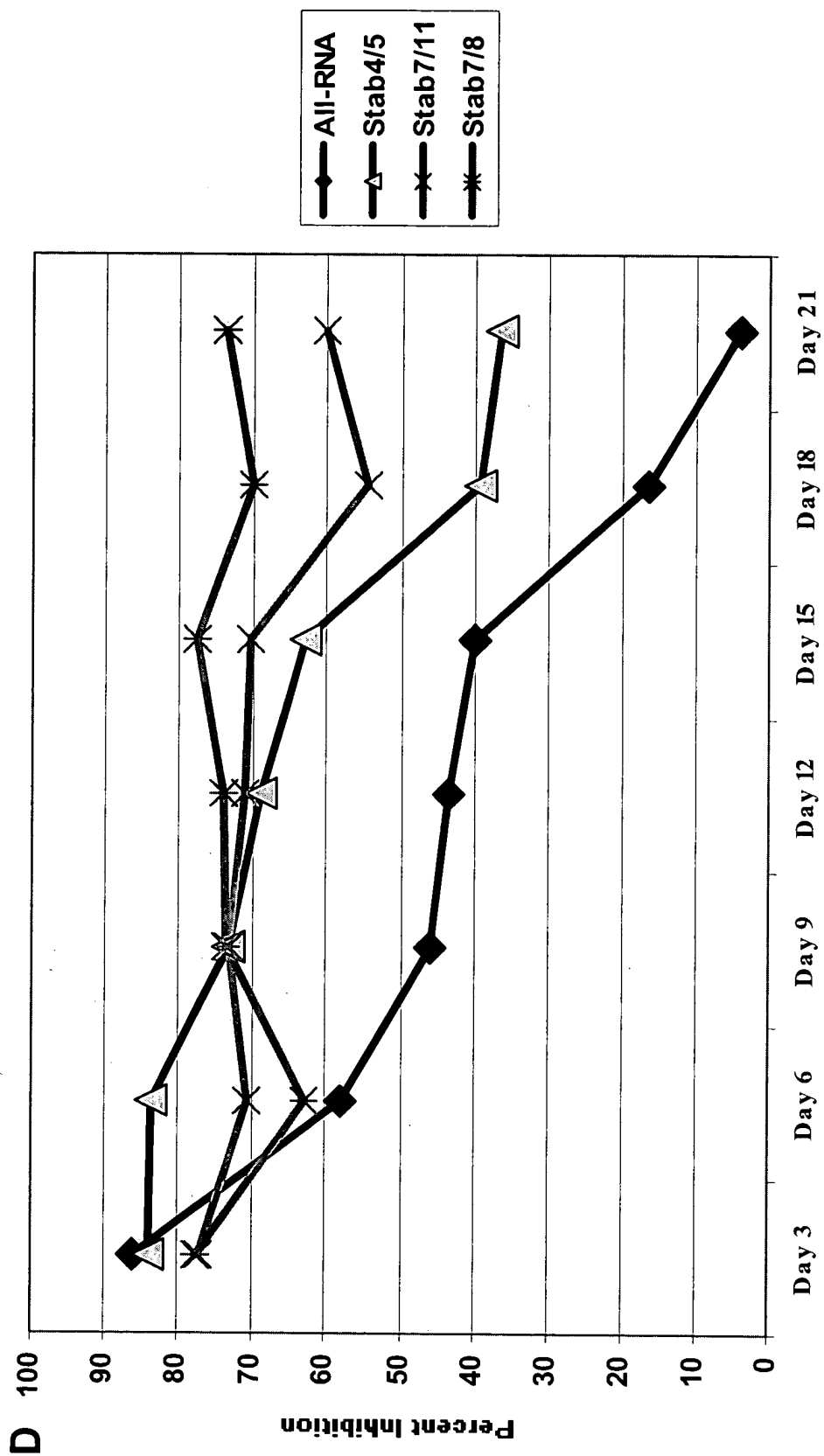
**Figure 77: Duration of Effect of Modified siNA Constructs**



**Figure 77: Duration of Effect of Modified siNA Constructs**  
 HBV siRNA Duration: Day 21

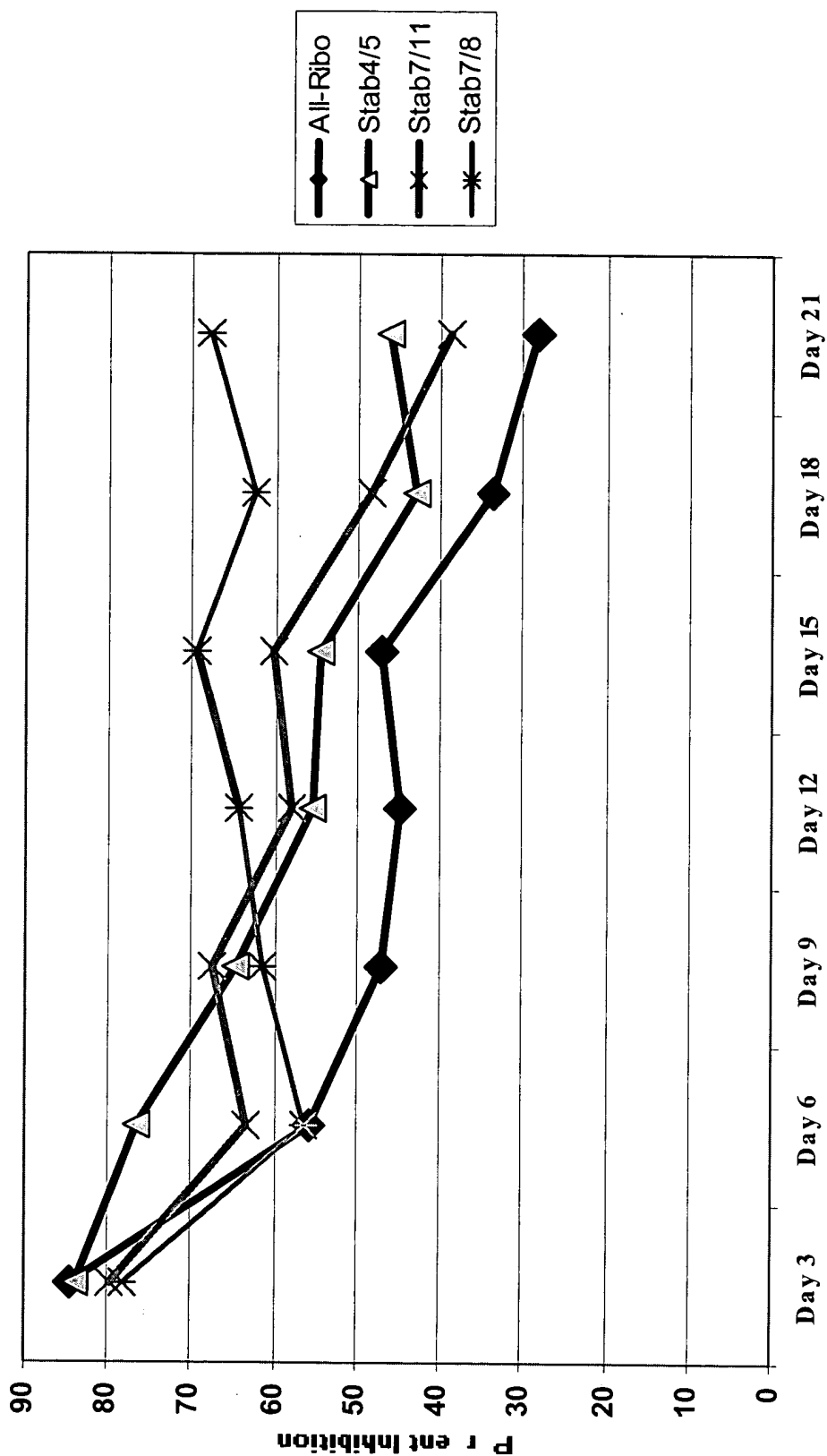


**Figure 77: Duration of Effect of Modified siNA Constructs**

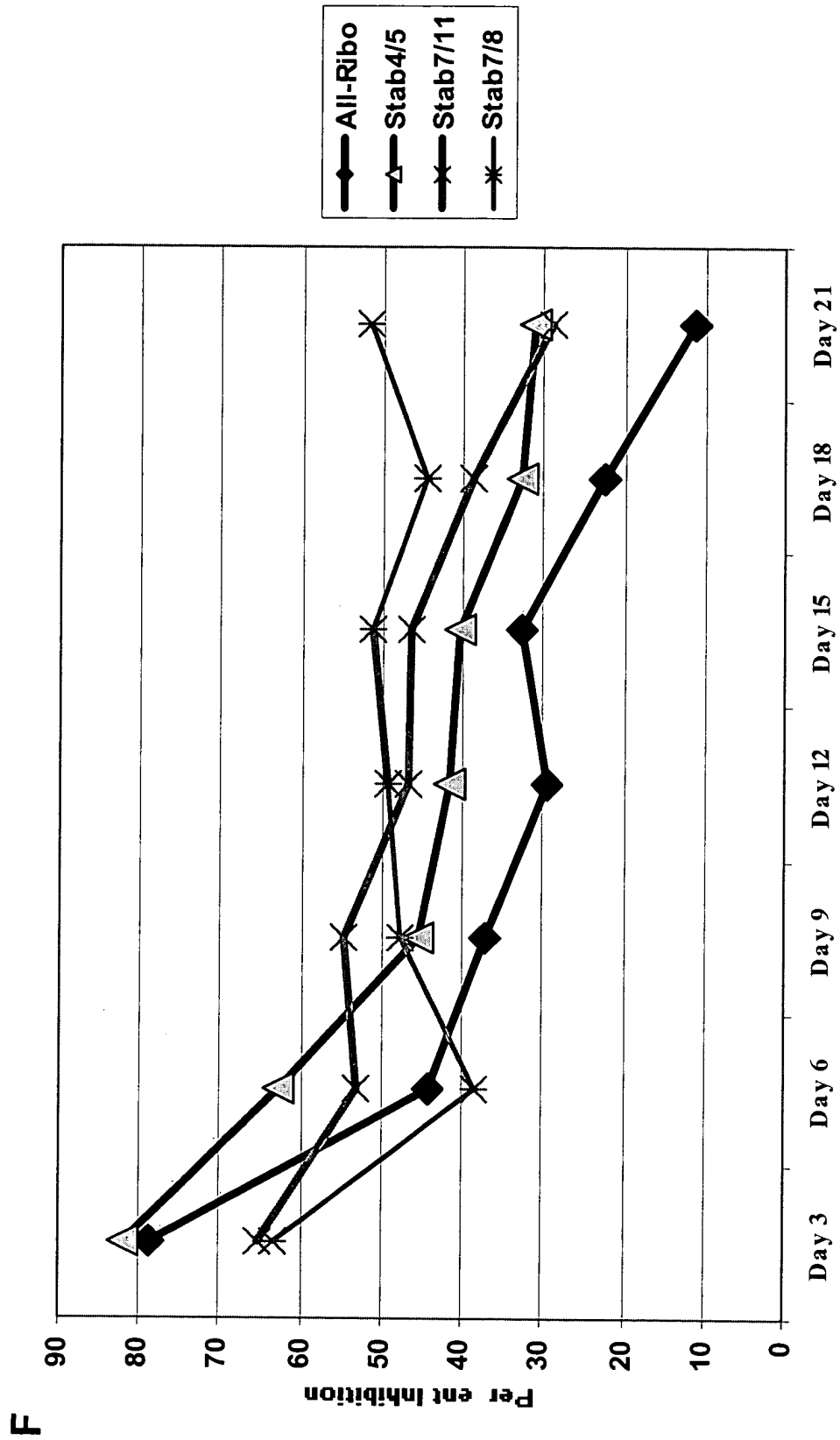


**Figure 77: Duration of Effect of Modified siNA Constructs**

**E**



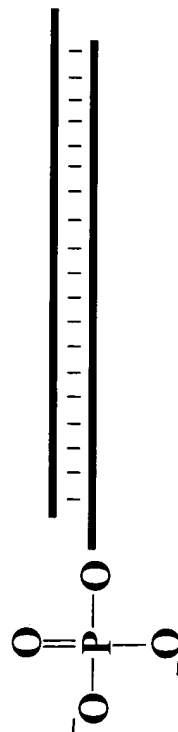
**Figure 77: Duration of Effect of Modified siNA Constructs**



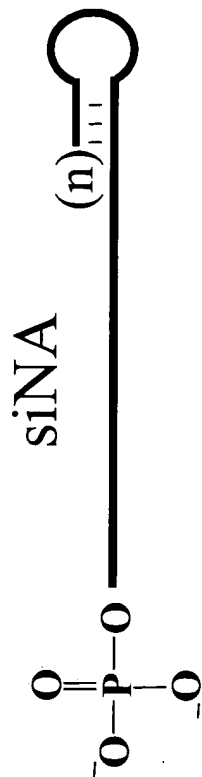
**Figure 78: Phosphorylated siNA constructs**



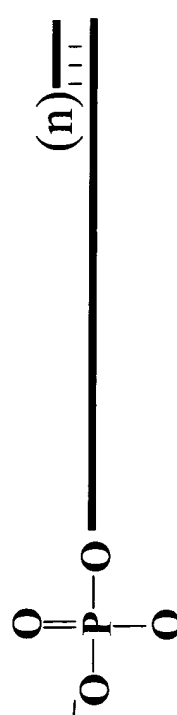
Phosphates can be modified  
as described herein



Asymmetric hairpin



Asymmetric duplex  
siNA



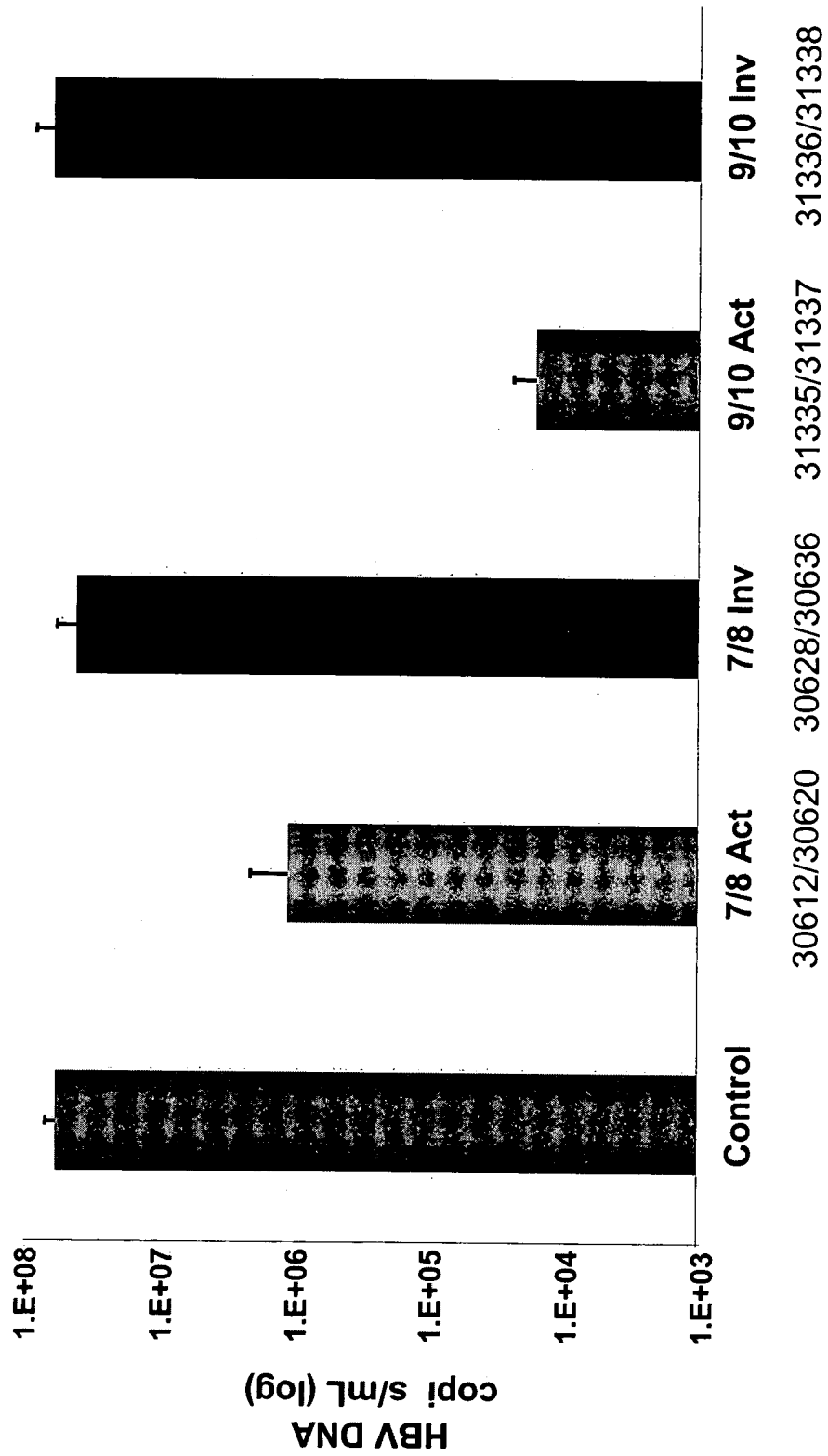
(n) = number of base  
pairs (e.g. 3-18 bp)

Chemical structures of various phosphonate and sulfonate groups, including their sodium and potassium salts, are shown. The structures include:

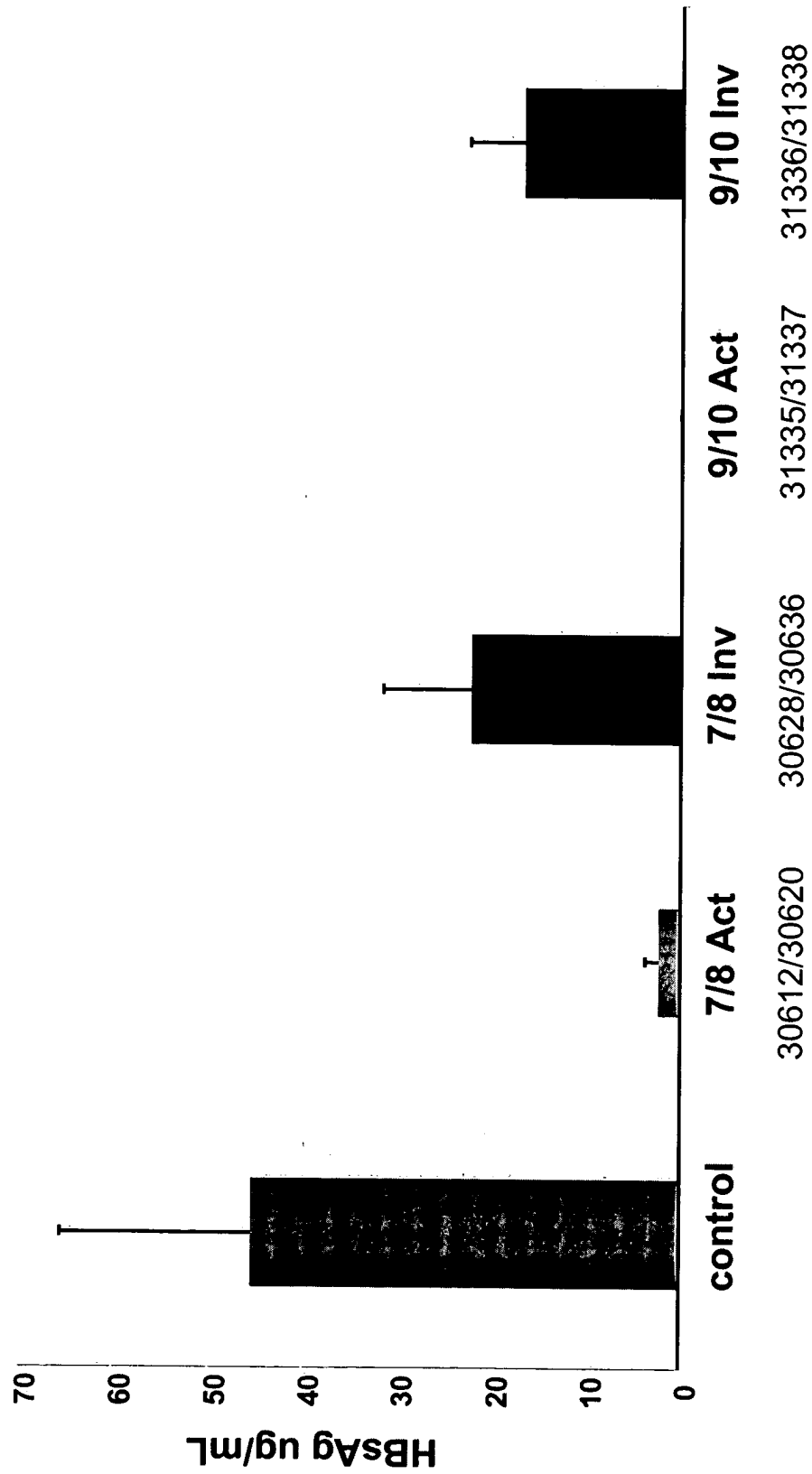
- Phosphonate groups:  $\text{P}(=\text{O})(\text{O}^-)_2$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{NH}_2)$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{CH}_2\text{NH}_2)$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{Me})$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{OMe})$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{Cl})$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{H})$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{CH}_2\text{SO}_3^-)$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{CH}_2\text{SO}_3\text{Na})$ ,  $\text{P}(=\text{O})(\text{O}^-)(\text{CH}_2\text{SO}_3\text{K})$ .
- Sulfonate groups:  $\text{SO}_3^-$ ,  $\text{SO}_3\text{Na}$ ,  $\text{SO}_3\text{K}$ .

The structures are arranged in a grid, with some groups enclosed in circles. The text "Sulfonic acid equivalent with Vanadyl equivalent with combination of other modifications herein" is present, indicating the relative reactivity of these groups.

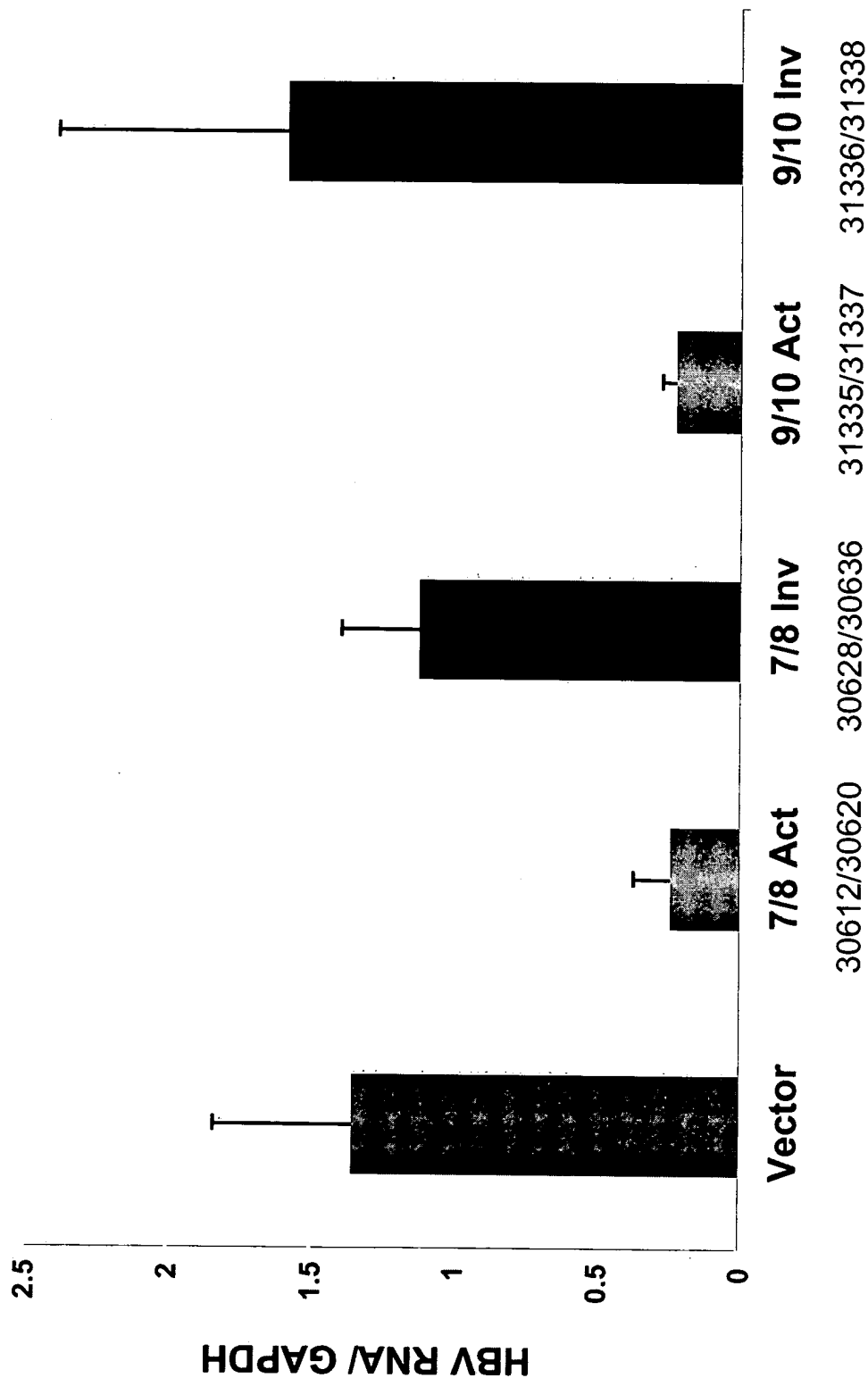
**Figure 80: Serum HBV DNA in Mice Treated with siNA Via HDI**



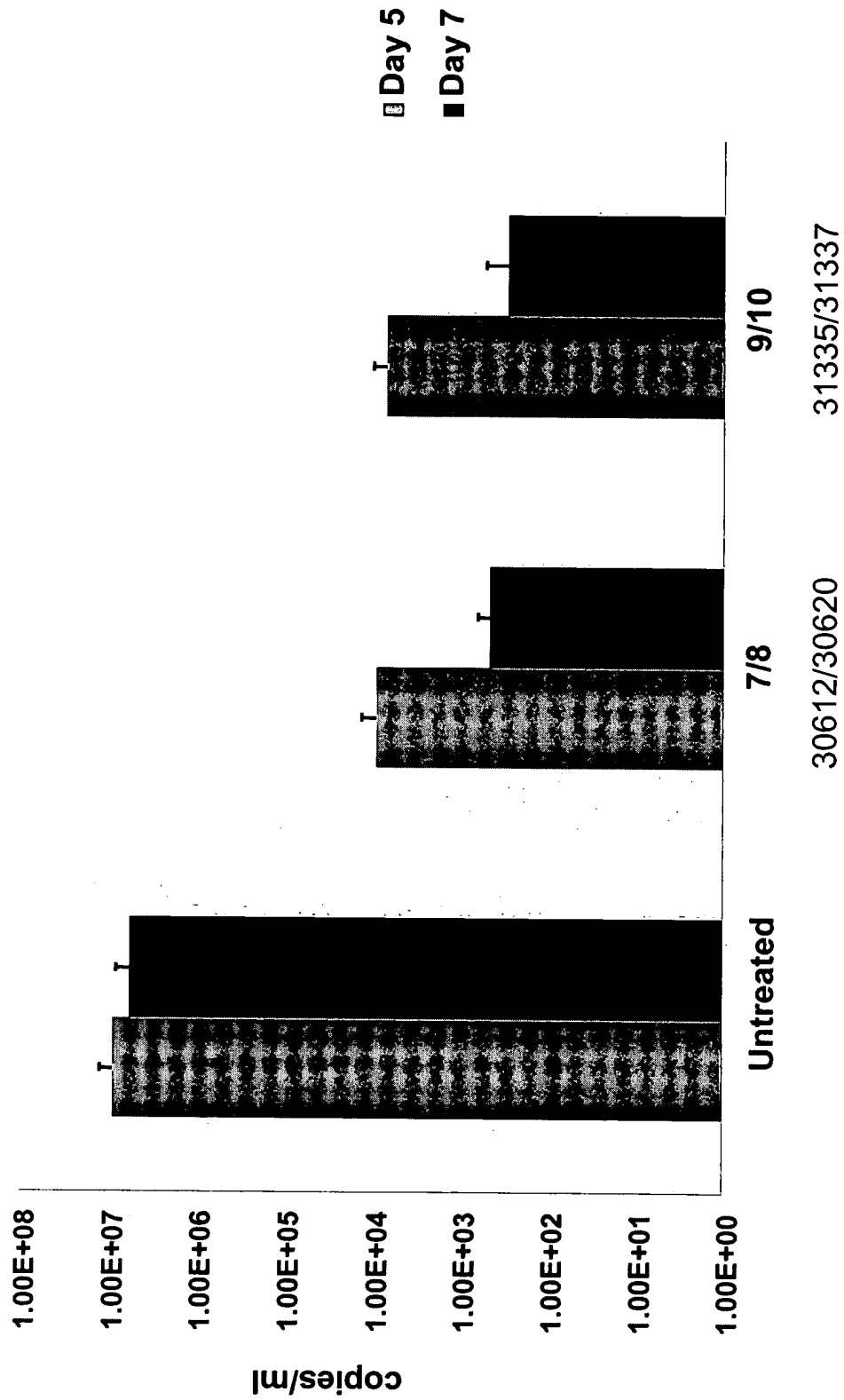
**Figure 81: Serum HBsAg in Mice Treated with siNA Via HDI**



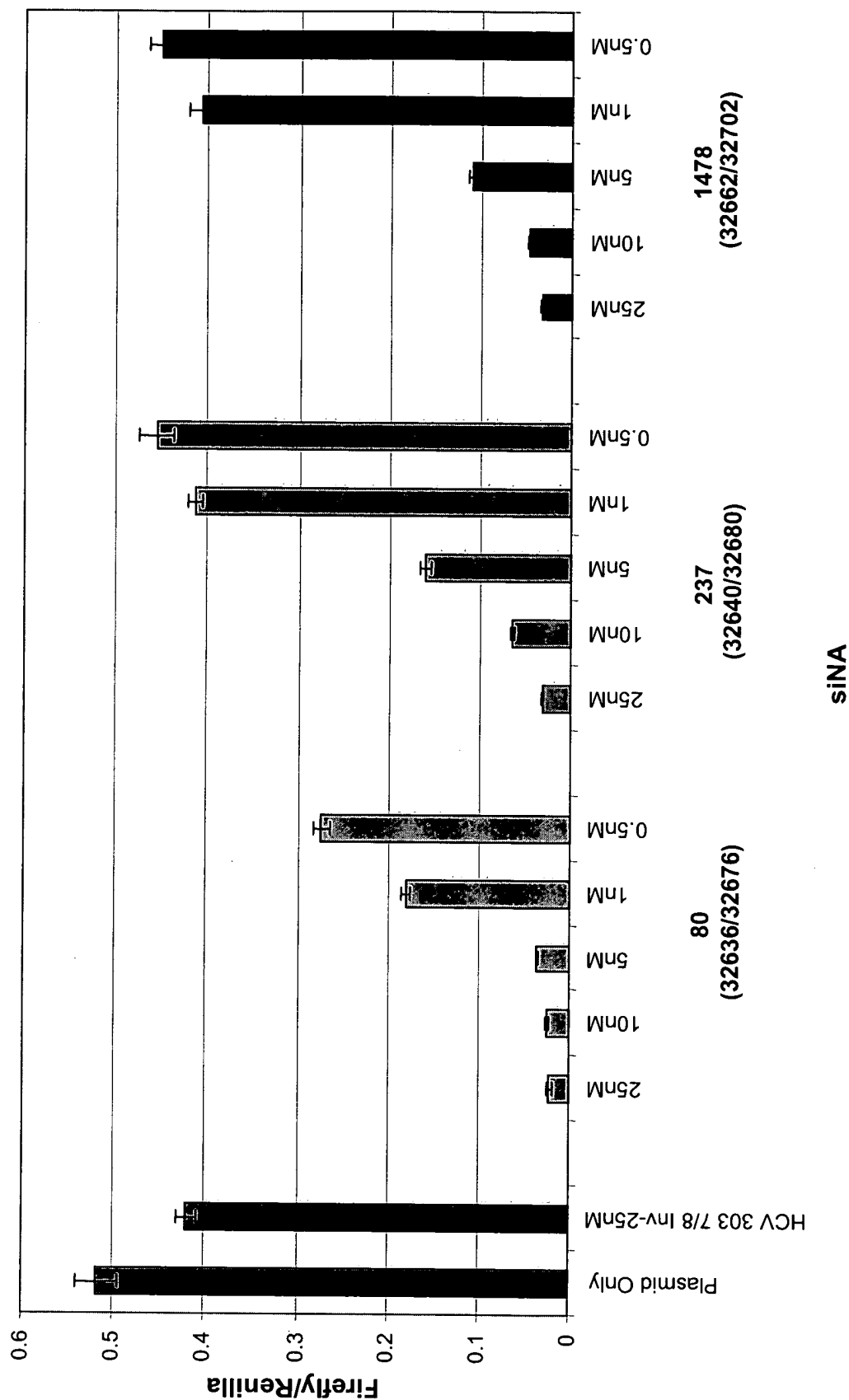
**Figure 82: Liver HBV RNA in Mice Treated  
with siNA Via HDI**



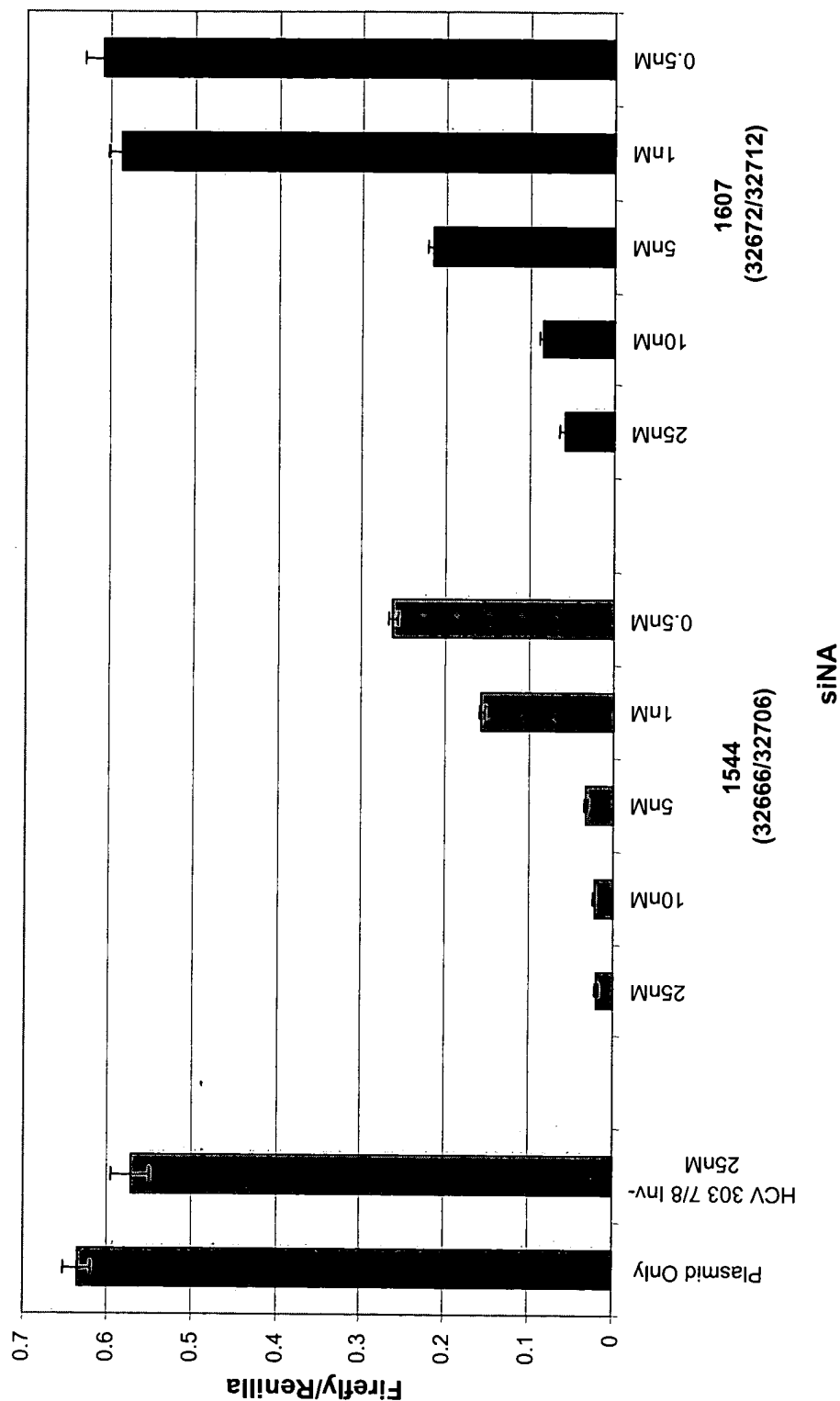
**Figure 83: Serum HBV DNA in Mice Treated with siNA Via HDI 5 and 7 days post treatment**



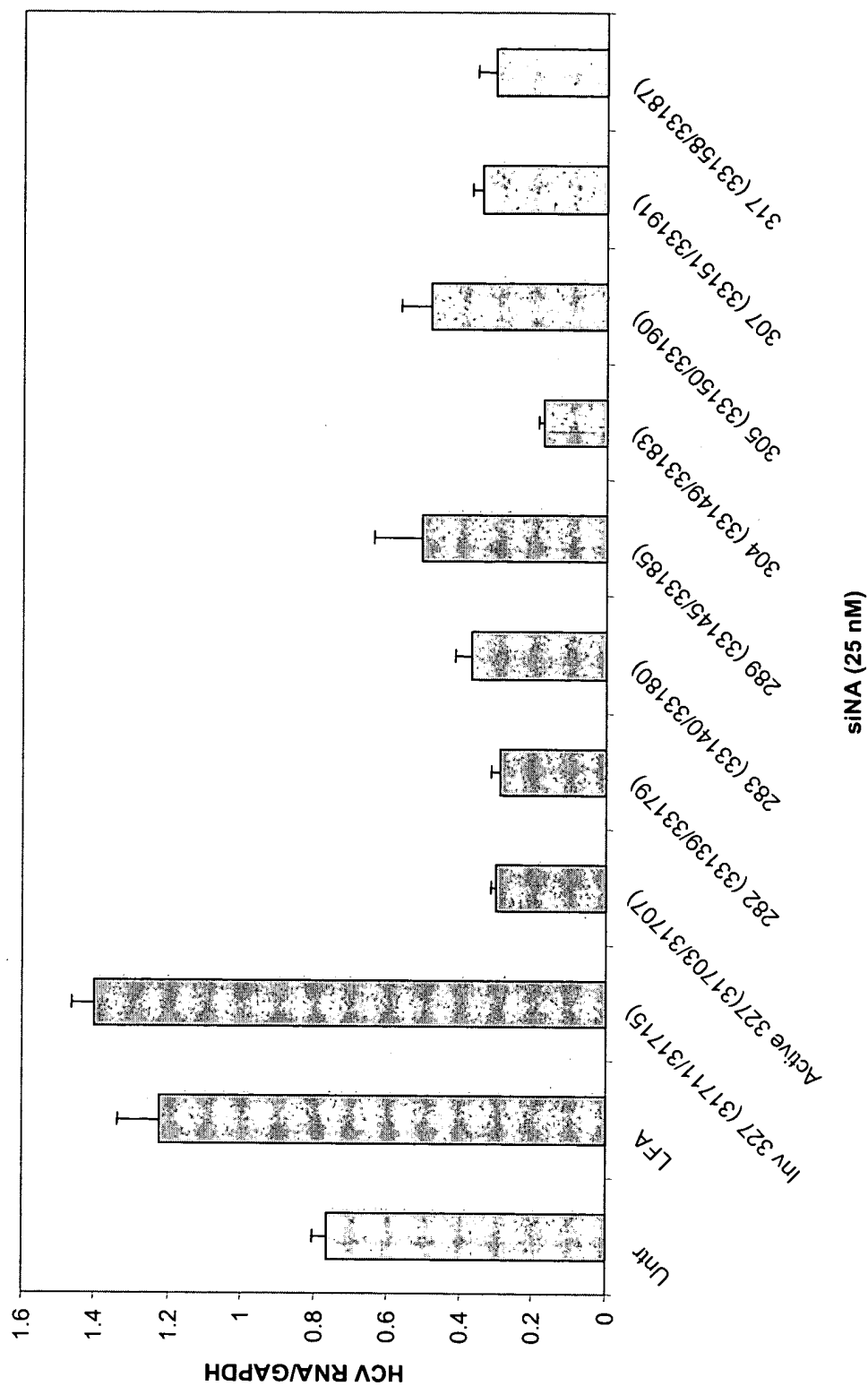
**Figure 84: Luciferase Dose Response  
 of select active siNA constructs**



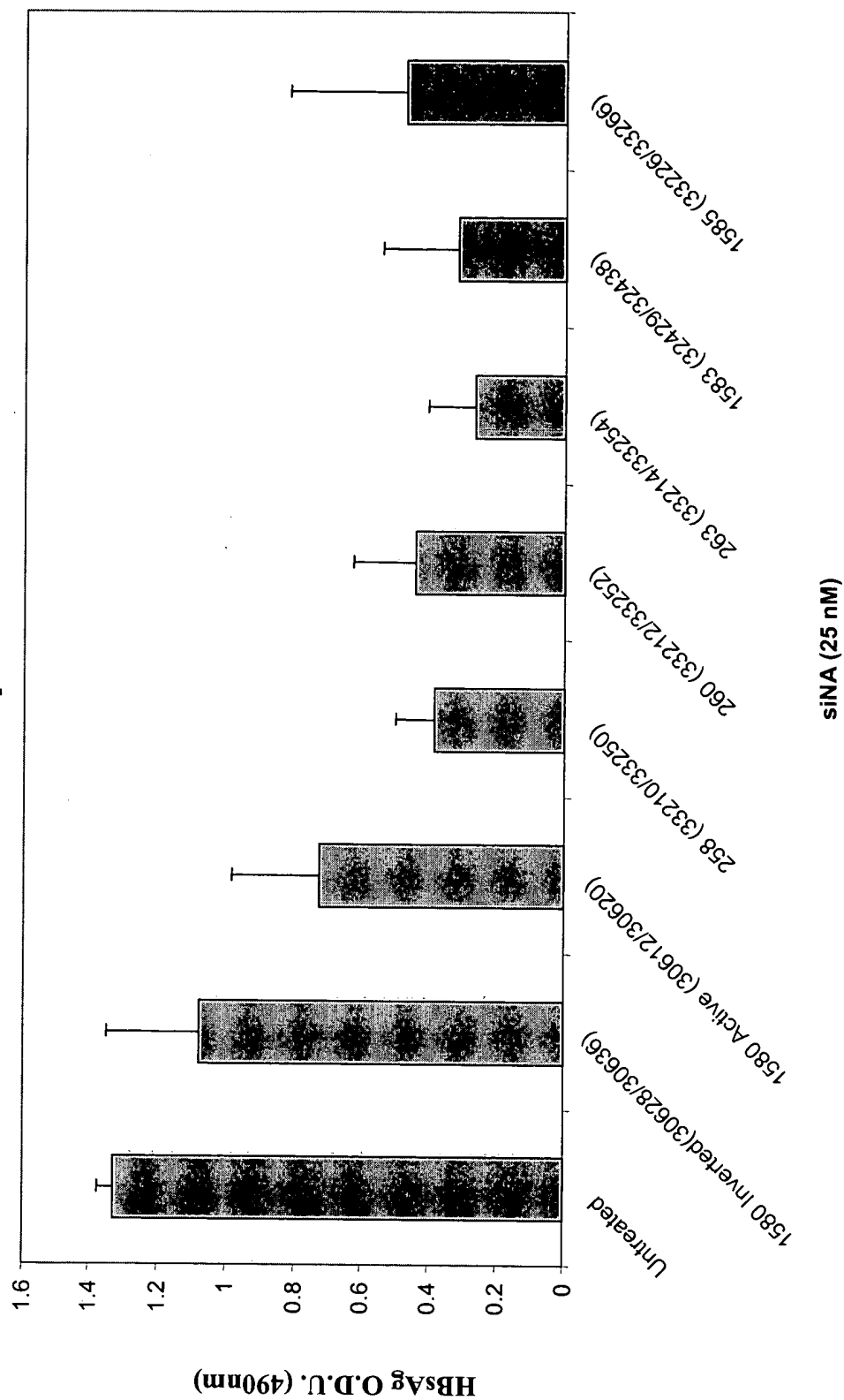
**Figure 85: Luciferase Dose Response  
 of select active siNA constructs**



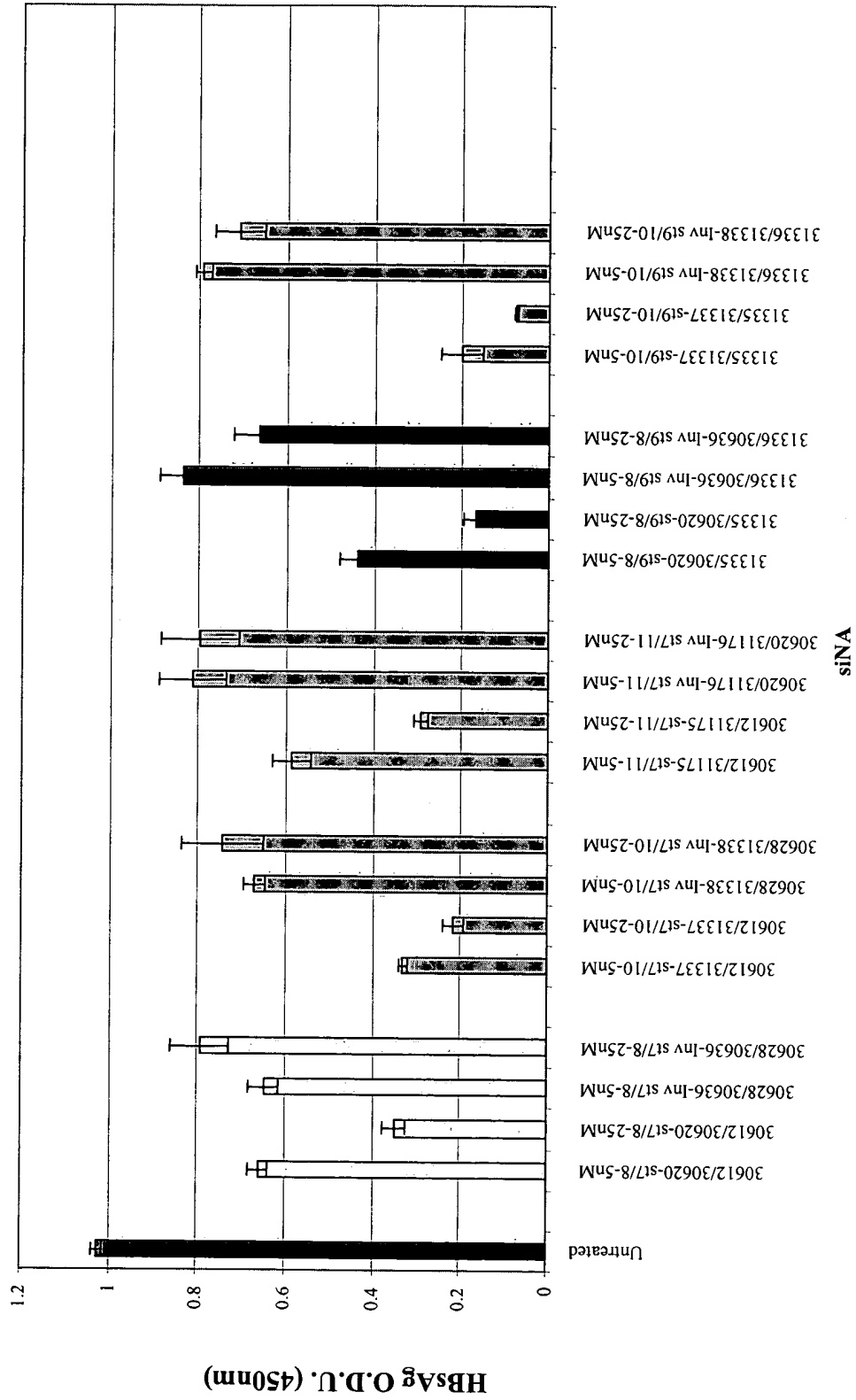
**Figure 86: Activity of Stab 7/8 Stabilized siNAs in HCV Replicon**



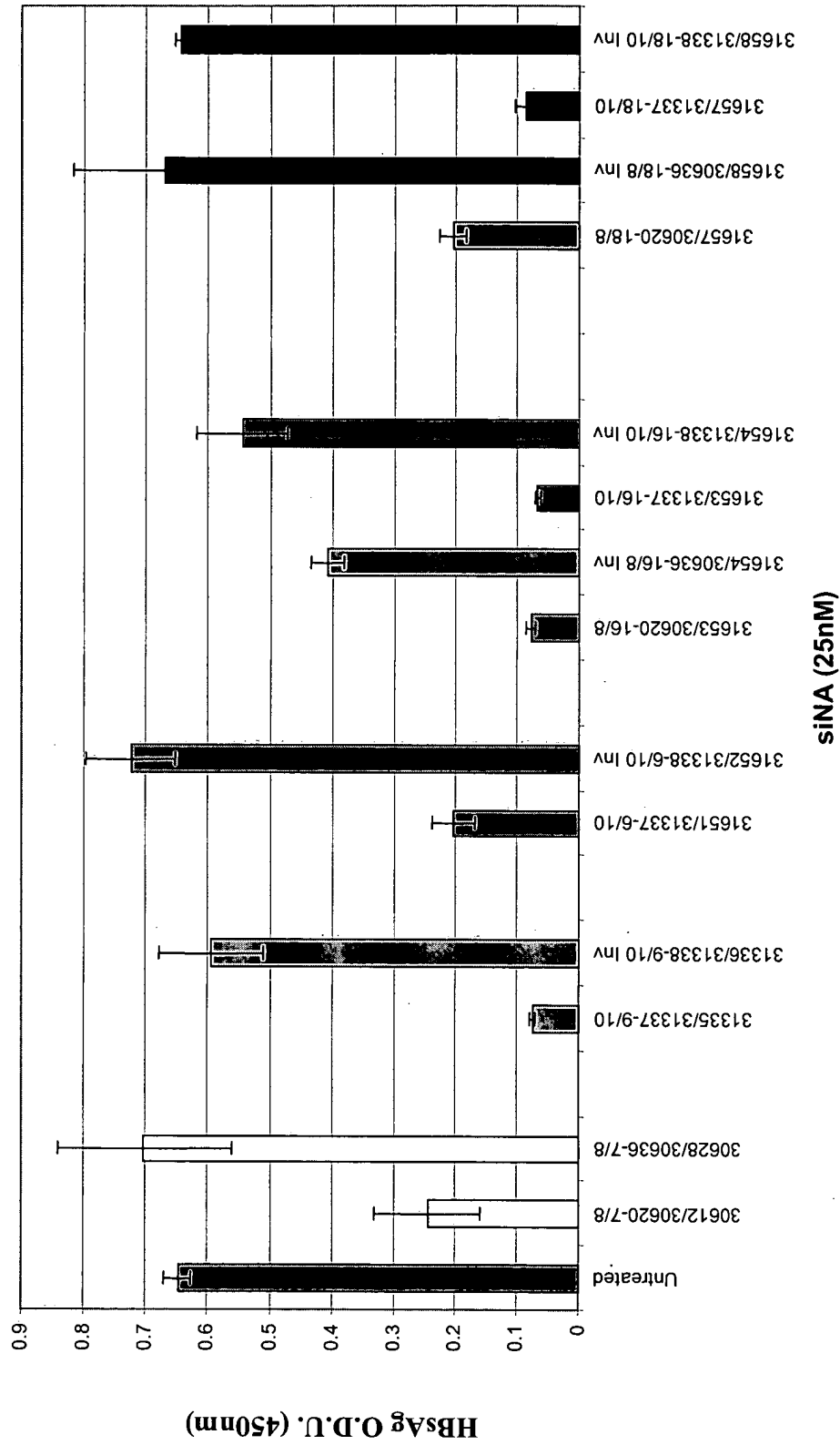
**Figure 87: Activity of Stabilized 7/8 siNAs Against HBV in HepG2 Cells**



**Figure 88: HBV/siNA to site 1580 Combination Constructs**



**Figure 89: HBV/siNA to site 1580 Combination Constructs**



*Figure 90: HBV/siNA to site 1580 Combination Constructs*

